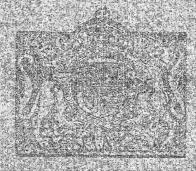
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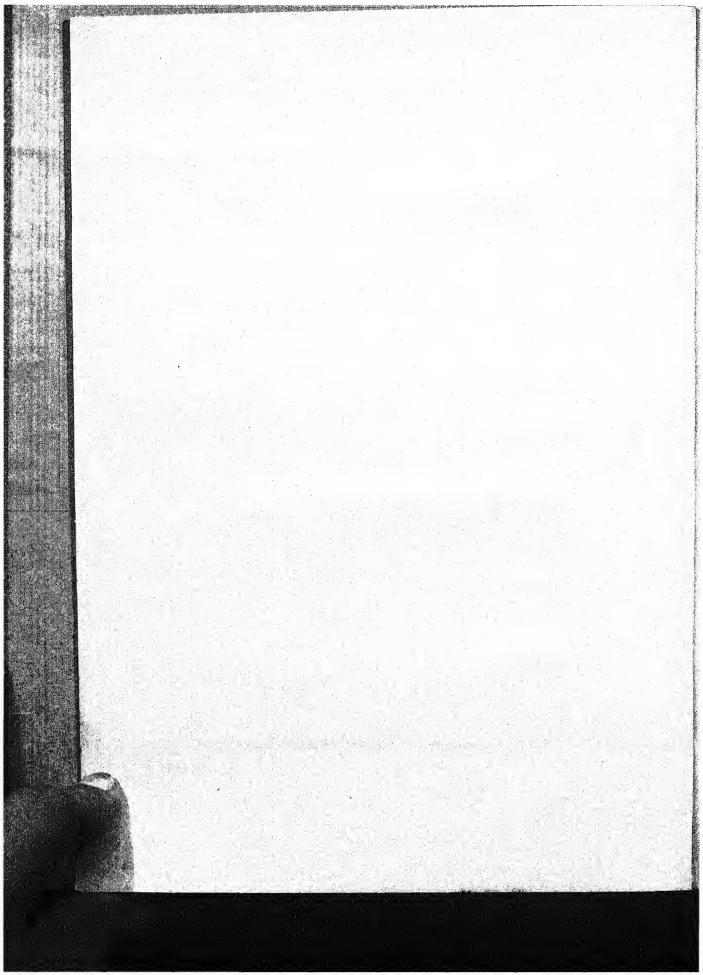
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# March 1934.

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# ORIGINAL ARTICLES

A COMPARATIVE STUDY OF SCHISTOSOMA SPINDALIS, MONTGOMERY, 1906 AND SCHISTOSOMA NASALIS, N. SP.

BY

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Lecturer in Parasitology, Madras Veterinary College.
(Received for publication on 27th October 1932.)
(With Plates I-V and five text-figures.)

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#### INTRODUCTION.

In the domesticated animals in India four species of Schistosomes are known to exist, one being S. bovis Sonsino, 1876, and the other three being S. bomfordi, S. spindalis and S. indicum discovered by Montgomery in 1906. All these Schisto-

somes are found in the portal veins or mesenteric veins or both, of cattle, sheep, goats, buffaloes and horses, as the case may be. In the Madras Presidency the author has met with only two species hitherto, viz., S. spindalis and S. indicum in the portal system of those animals and, so far as he is aware, there appears to be no record of any other species having been found excepting the nasal schistosome recently discovered in the veins of the nasal fossae of cattle.

Twelve years after Montgomery [ 1906 ] published his paper on those three Schistosomes discovered by him, the life-history of one—S. spindulis—was worked out by Liston and Soparkar [ 1918 ]. Almost another decade elapsed before Fairley and Mackie (1925) studied the histopathology of S. spindalis in goats and proved that it affects exclusively the portal system and its ova are voided only with the faeces. Liston and Soparkar [ 1918 ] also found that the ova of S. spindalis pass out in faeces of the infected animal. The other two Schistosomes of cattle discovered by Montgomery have so far been found to develop only in the portal system; consequently their ova are found in the faeces of the host. As regards S. bovis, it has been proved by MacHattie and Chadwick [1932] and others that it chiefly inhabits the portal system and the ova of these find an outlet only through the faeces of the host. In man, two out of the three species, develop in the portal system and the only exception being S. haematobium (Weinland 1858) which develops usually in the pelvic plexus and its ova are voided chiefly in the urine. It is interesting to note that in nasal schistosomiasis in cattle the species of Schistosome concerned chooses the nasal veins as its habitat and its ova are found only in the nasal discharge, a departure analogous to that of S. haematobium in man. In these two species, therefore, the site of infection in their normal hosts is different from that of the other species found in man or cattle.

Sometimes two or more species of Schistosomes are found in the same host. Montgomery [1906], for example, found S. bomfordi and S. spindalis in the same animal and he was able to distinguish the one from the other by noting the difference in the morphology of the worms and by the shape of their ova. Leiper [1915] writes—"A comparative study of mature specimens of S. bovis and S. haematobium showed that those two species were so closely allied that when experimentally reared in an abnormal host, a differential diagnosis could only be made with certainty upon the characters of the fully grown worms and especially by those of the egg shell". The last remark of Leiper is further strengthened by MacHattie and Chadwick [1932], who say that "the eggs of the various Schistosomes from man and animals, within certain limits of variation, have a definite shape which is generally regarded as being characteristic of the species". It would appear, therefore, that the morphology, the characteristic shapes of the ova and the site of infection are important

points in arriving at an identification of the species of Schistosomes. In addition to this, the morphology of the miracidium, and cerearine should also help in the diagnosis, where the life-history of the worm is known.

Bhalerao [ 1932 ], in the course of his paper observes that:-

- (1) much importance cannot be attached to the question of the number of testes present in the male schistosome.
- (2) the length of the common caecum cannot be taken into account owing to its variable nature.
- (3) the dimensions of the body cannot be given any importance since they vary with age.
- (4) the tuberculate or non-tuberculate nature of the cuticle should not be considered as of any value in distinguishing one species of schistesome from another.
- (5) polymorphism in eggs appears to be the rule in many species of schistosomes.
- (6) the site of infection does not help matters.

It would appear therefore that the specific diagnosis of a schistosome has no value, when it is based on the above points. Hence Bhalerao's diagnosis of the Nasal Schistosome as no other than S. spindalis Montgomery [ 1906 ] cannot be accepted in the light of his own observations, since he arrives at a diagnosis by comparing, in a tabular form, some of the features enumerated above. Three other workers, however, consider that the nasal schistosome is different from S. spindalis. Datta [ 1932 ], says that the parasite resembles S. spindalis, but certain differences suggested to him that it may be a new species. Malkani [ 1933 ], thinks that it is a variety of S. spindalis. Rao [ 1932 ], describes it as a new species. Now that the life-history of the nasal schistosome has been worked out by Rao [ 1933 ] and that of S. spindalis has already beed worked out by Liston and Soparkar [ 1918 ], it would be interesting to compare these species from different standpoints, such as the shape of the egg, morphology of the miracidium, cerearia and of the worms to arrive at a specific diagnosis.

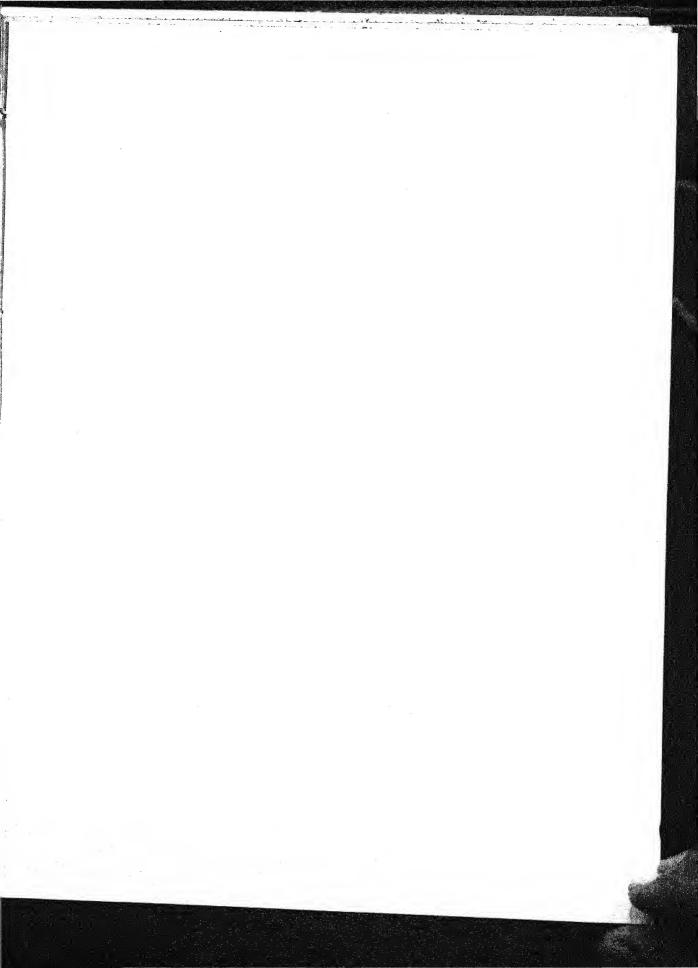
#### THE OVA.

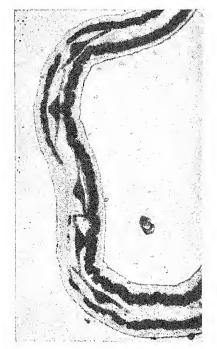
The author had opportunities to study the ova of Nasal Schistosomes in the nasal washings obtained from over 300 cases from different parts of the Presidency

and also from three experimentally infected calves. The shape of the ovum is an elongated spindle. The middle third of the body of the ovum, which is concavoconvex, is prolonged into a horn on either side, one of which has its extremity bluntly rounded and the other terminates in a spine. The spine is straight in some ova, but in the majority of mature ova, it is curved slightly or sharply like a hook, towards the concave margin of the body. In none of them, however, was the spine seen curved towards the convex margin of the body of the egg. The horns themselves are either straight or slightly turned up towards the convex margin of the egg. With proper orientation the typical shape of this "zygomorphic" ovum is always seen. In the fresh nasal discharge, the mature ovum shows a miracidium inside it and it appears that in the majority of the ova the snout of the miracidium is pointed towards the spine end of the ovum. In the egg, the miracidium occupies only the central area and the horns of the egg seem to contain a clear fluid. This fluid in the horns of the egg becomes apparent when some tap water is added to the fresh nasal washings. A seething mass of minute bubbles arise in this fluid round about the miracidium. (Pl. III, fig. 4.) Presumably, an increase of these bubbles brings about a rupture of the egg shell, liberating the embryo. confirms Malkani's [1933] observations regarding this phenomenon.

The ovum of S. spindalis is also spindle-shaped, zygomorphic and has a body and two horns. In this ovum there is a bulge on one side of its body and the opposite side is straight throughout its length or even slightly convex at its middle. The spine is straight or curved towards the straight border and in over 15 per cent. of these ova, the spines are curved upwards towards the convex side of the body of the egg. The horns are usually straight and in a few ova they are slightly inclined towards the straight side and they are never seen to bend towards the bulged side of the egg as in the ova of nasal schistosomes.

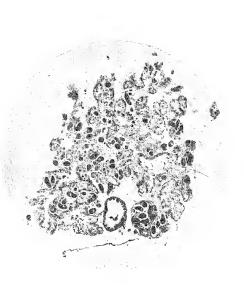
Datta [1932] has published a photograph of a typical ovum from the nasal washings and in that the bend of the horns towards the convex side of the body is clearly seen. Malkani [1933] says that the ovum found in the nasal washings has a concave border in place of the straight border found in the ova of S. spindalis. He has also given a photograph showing the typical shape of the ova. Rao [1932] has published a photograph of the ovum of S. spindalis and another of the nasal schistosome to show the difference in their shape and size. Bhalerao [1932] has illustrated his paper with drawings of ova in nasal washings and admits that they usually possess, more or less, a degree of curvature on one side. He also says that the egg of nasal schistosome presents different appearances when viewed in different positions, and this is to be expected in a zygomorphic ovum. This is also true with the ovum of S. spindalis.



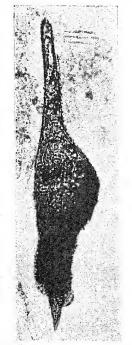


3. Ova of S. spindalis in uterus. Note shape,  $\times 60$ . Photograph of fresh material.

1. Ovum of S. spindalie. Note the shape and actino-body round the hom/behind the spine.



4. Section of liver of experimentally infected snail showing cercariae in sporocysts.



2. Ovum of S, nasalis in uterus. Note shape,  $\times 60$ . Photograph of fresh material.

Montgomery [1906], Tanabe [1925], Baylis [1929] and Faust [1930] have shown, in their drawings of Schistosome ova, that one side of the ovum of S. spindalis is flat. Fairley and Mackie [1930] have given a diagram of a female S. spindalis with ova in its uterus and also of a few mature ova. These illustrations show that one side of the ovum is flat or even slightly convex and the other side is bulged in the middle. In some of these ova the spines are curved towards the bulged border. Photo-micrographs (Pl. I, figs. 2 & 3) of fresh female schistosomes show the difference in shape and size of the ova in utero. Thus there appear to be differences in the shape of the ova of these two species, and these are tabulated below for comparison.

TABLE I.

en entiro en en		-		Ova of S. spindalis.	Ova of nasal schistosome.
Length*	•	•		284—400	, 336—581
Breadth	•	•		8090	60—80
Spine .			٠.	Often curved towards the bulged border.	Often curved towards the concave border.
Horns .		•	•,	Straight or slightly curved towards the flat side.	Straight or slightly curved toward the bulged border.
Base* .	,		•	Flat or slightly convex in its middle.	Concave.
Where found	•		•	Only in fueces	Only in the nasal discharge.

<sup>\*</sup> The length and breadth are in microns and include the maximum and minimum given by various authors. The base represents the side appears to the bulged margin of the body of the egg.

Datta [1932] says that the type of ova met with in the nasal discharge has not been found in the faeces or urine of animals having nasal schistosomiasis. Malkani [1933] confirms this. The author examined samples of faeces and urine of 86 cases, and in addition he has frequently examined the faeces and urine of the three experimentally infected calves and agrees with Datta and Malkani. Further the author did not find ova typical of S. spindalis in the nasal washings. Therefore even in the same animal, it would appear that the two species of Schistosomes with the two different types of ova, are met with in two different situations respectively. From the table of differences it is evident that the ovum of the nasal schistosome is different from the ovum of S. spindalis.

Malkani [1933] says that he found in the nasal washings only one type of egg with one typical shape thus confirming Rao's [1932] finding. Since then the author has examined a large number of specimens of nasal discharge, preserved in 3 per cent. NaOH or KOH solution, received from the districts and also fresh nasal washings from the three experimentally infected calves. None of the ova showed any departure from the typical shape described above. Occasionally some immature ova showed a narrower contour and a smaller size than the rest. Even in the sections of tissue the typical shape of the ova is seen provided the ovum is cut longitudinally about its middle. Datta [1932], however, met with a few ova having peculiar shapes, with or without spines or even having two spines one on either end. These ova have been illustrated by Bhalerao [1932]. These shapes do not appear to be within reasonable limits of variation in a given species and await confirmation.

Bhalerao [1932] suggested that the central bulge on one side of the ovum of nasal schistosome is probably due to some organ of the developing embryo. The present author has verified this and found that the bulge in the body of the ovum of either the nasal schistosome or of S. spindalis is not caused by the developing miracidium or any of its organs. The centrally placed miracidium in an egg has failed to show any protrusion of its part or parts beyond its usual almond-shaped contour during its development and apparently the miracidium is only a passive entity within the characteristically shaped shell moulded in the ootype. It has also been seen that even the active movements of a miracidium inside the egg, have no effect whatever even to temporarily alter the shape of the rigid egg shell.

## THE MIRACIDIA.

In the fresh nasal discharge, particularly in that from recently infected animals, fully developed miracidia are seen in the majority of the ova. Often free miracidia are encountered in the undiluted discharge particularly when the discharge is thin or if it is kept in a tube for some little time before examination. This confirms Malkani's [1933] finding of free miracidia in fresh nasal discharges.

The mature ova of S. spindalis obtained in the rectal scrapings, on the other hand, do not seem to rupture unless water is added to the sample, when bubbles develop inside the ovum, just as in the case of nasal schistosome ova, before the shell bursts.

The miracidium of the nasal schistosome, on emerging from the egg, swims about vigorously in water with the aid of cilia present on its cuticle. It appears to

be positively phototrophic and can live in tap water up to about 24 to 36 hours at laboratory temperature. The shape of the miracidium is more or less like that of an almond with its shoulders slightly broadened at the anterior extremity, and it appears to be slightly flattened dorsoventrally. The anterior extremity bears on it, a protrusible semi-circular suout without any cilia. The posterior extremity is bluntly rounded and is covered over with cilia as on the other parts of the cuticle. Cilia are absent round two papillae, one on each shoulder, and each of these papillae is the common opening of the ducts of a cluster of small secretory glands which will be referred to later. About the middle of the body of the miracidium is a girdle of groups of cilia running right round the body. A little behind this girdle, around each of the two excretory pores, cilia are absent. The girdle of groups of cilia is an interesting morphological detail. This is seen when the surface of the cuticle is carefully brought into focus of the microscope and it is best seen when the ciliary movements are slowing down. In the miracidium of the nasal Schistosome the girdle is formed by 10 to 12 groups of cilia across the visual section, and each group is cone shaped.

On the semi-circular snout is the small oral opening which leads into a short tubular or saccular gut containing granular material. The gut extends backwards along the median line, to a rounded bunch of small secretory glands or may even slightly overlap this organ. On either side of the gut is a long and fairly large secretory gland, containing large oxyphilic granules, the duct of each opening on the postero-lateral margin of the snout. These glands, which may be called the anterior or lateral secretory glands, are very much longer than the gut. Behind the gut, in the median line is a fairly large circular looking object consisting of a group of small secretory cells or glands showing fine basophilic granules. This group appears to be a pair placed close together. The ducts of each pair of groups open on a peg-like papilla on each shoulder of the miracidium. Surrounding these two groups of secretory cells is the nerve tissue with a few short linear prolongations proceeding posteriorly into the parenchyma of the miracidium. In the body of the miracidium, particularly in the posterior region, there is a fairly large number of rounded germinal cells. These germinal cells, apparently, are derived from the germinal epithelium lining the inside of the cuticle,

The excretory system consists of an anterior and posterior flame cell in each mesial half of the body. One flame cell is situated just near the rounded group of small secretory cells and from it a collecting tubule arises to join a similar tubule, which takes its origin from the posterior flame cell situated near the excretory pore. Each of the common tubes formed by the junction of these two tubules, at first passes forwards, re-curves on itself, proceeds backwards and at about the middle of

the body it forms a tangle and ultimately opens in the excretory pore, which is at the margin of the commencement of the posterior third of the miracidium. (Text-fig. 1.)

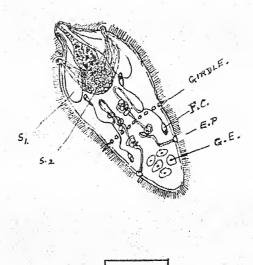
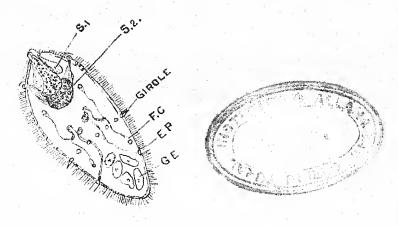


Fig. 1. Miracidium Schistosoma nasalis.

0.05

The miracidium of S. spindalis has not been fully described hitherto and this opportunity is taken to do so to facilitate comparison. The miracidium of S. spindalis is also almond shaped, has a ciliated coat and appears to be smaller in size than the other one. The snout is smaller and the oral opening leads into a tubular gut containing granules as in the other. On either side of this gut is a short saccular anterior secretory gland, shorter than the gut and contains oxyphilic granules. The duct of each of these glands opens in the same situation as in the other. Behind the gut in the median line the two groups of secretory cells are exactly as in the other miracidium and their ducts open on the two peg-like papillae as in that one. The arrangement of the nerve tissue and the excretory system is similar to that of the other miracidium but the common excretory tubes do not proceed forwards to such an extent and are less convoluted. There are breaks in the ciliary coat only around the secretory and excretory pores and a girdle of ciliary groups is present. It is interesting to note that the number of groups of cilia on this girdle varies from 6 to 8 across the visual section. The body contains larger and more or less quadrangular

SCHISTOSOMA SPINDALIS, MONTGOMERY, 1906 & SCHISTOSOMA NASALIS, N. SP. germinal cells when compared to the other. (Text-fig. 2). The differences between



0.05

Fig. 2. Miracidium of Schistosoma spindalis.

Secretory glands.

Flame cell.

Excretory pore, Germinal E. P. G.E. epithelium.

the two miracidia are summed up in the following table: --

TABLE II.

What is proposed to the state of the state o	Miracidium of S. spindalis.	Miracidium of the nasal schistosome.	
Anterior exerctory glands     The gut	Shorter than gut Langer than the anterior secretory glands	Longer than gut.  Shorter than the anterior secretory glands.	
3. No. of ciliary groups on the girdle 4. Germinal cells	6 to 8  Quadrangular and large	10 to 12. Rounded and small.	

# THE INTERMEDIARY HOST.

Rao [1933] successfully infected three calves with Cercariae Indicae XXX Sewell 1922, which he obtained from two species of snails, viz., Limnea leuteola (Lamarck) and Planorbis exustus (Deshayes). He observed that the infection rate was more in Limnea species than in Planorbis species. Sewell [1922] found that Cercariae Indicae XXX predominated in Limnea accuminate than in Planorbis exustus. As regards the cercariae of S. spindalis, Soparkar [1921] has stated that these were always found in Planorbis exustus and very rarely in Limnea accuminata. Fairley and Mackie [1930] obtained cercariae of S. spindalis only from Planorbis exustus in Bombay for their work. If this has any significance, then it shows that the Nasal Schistosome prefers Limnea species as its intermediate host and S. spindalis chooses Planorbis species. It is evident, therefore, that the Nasal Schistosome is less selective in choosing its intermediate host than S. spindalis.

#### THE CERCARIA.

The cercaria concerned is small, apharyngeal, and brevifurcate. It is a feeble swimmer and in a test tube of water its movements are mostly up and down. It swims with its tail foremost and is positively phototrophic. The body surface is covered over with small thin spines pointing backwards. The tail stem and furcalrami are covered over with coarser spines comparatively and these are curved slightly at their ends.

The body of the cercaria is pearshaped and on its wider end the forked tail is inserted. In the anterior end of the body is a muscular and oval penetrating organ crowned with ten hollow spines, five on each side of the median line. The ventral sucker is situated at the commencement of the posterior fourth of the body and is lined with three or four rows of fine spines. When the ventral sucker is held contracted, it usually shows a "Y" shaped depression on it.

The alimentary system consists of an oral opening situated subterminally on the penetrating organ, leading into a fine tubular œsophagus which ends in a dilated sac in front of the ventral sucker.

The secretory system consists of five pairs of secretory glands arranged on either side of the ventral sucker and in the space between it and the posterior end of the body. On each side, the anterior pair of glands are filled with coarse granules and the posterior three pairs contain fine granules. Their ducts open individually in the pointed hollow spines referred to already.

The genital system is represented by a group of cells immediately behind the ventral sucker. A few cells in the posterior and lateral margins of the body are seen and apparently these are parenchymatous cells and not rudiments of testes as Sewell [1922] considers.

The excretory system is composed of a small round bladder situated at the junction of the body and the tail. From the bladder two canals proceed forwards,

one on each side, between the secretory glands and the cuticle. About the middle of each main canal, a short length of the wall, which appears to be rigid contains two patches of cilia. Each of the main canals, after reaching the level of the ventral sucker, bifurcates into two tributary canals, one of which proceeds anteriorly and the other posteriorly. The anterior one passes forwards and near the anterior third of the body it bifurcates again into two fine cellecting tubules each ending in a flame cell, one cell being near the middle of the body and the other just behind the penetrating organ. The posterior tributary canal proceeds backwards, parallel to the main canal, and at about the level of the bladder bifurcates into two fine cellecting tubules. One of these fine tubules is short and ends in a flame cell near the bladder, and the other fine tube passes into the tail stem and ends in a flame cell. The flame cell formula can be summarised as follows:—

$$2 \times [2+1+(1)] = 6(+2).$$

Thus it is seen that this cercaria has three pairs of flame cells in its body and one pair in the tail stem.

The caudal excretory canal arises at the posterior opening of the bladder, the so-called "eyelet" aperture, and passing along the whole length of the tail stem, it bifurcates into the rami and ultimately each opens into a funnel shaped excretory pore situated at the end of each furcal ramus.

The cercaria conforms to the description of Cercariae Indicae XXX Sewell, 1922 and it is concluded that both are identical. (Text-fig. 3).

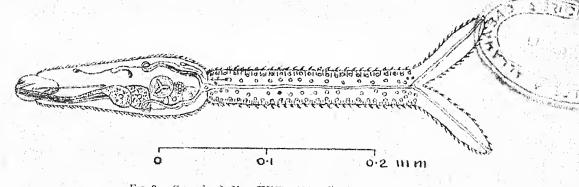


Fig. 3. Cereariae Indicae XXX. (After Sewell.)

The cercaria of S. spindalis, which has been described by Soparkar [1921], is bigger in size than the one described above and the most important difference is that this cercaria has four pairs of flame cells in its body and one pair in the tail stem. Its flame cell formula can be represented thus:—

$$2 \times [2+2+(1)] = 8(+2)$$
.

The comparison of the flame cell formulae of both at once suggests that the cercaria of S. spindulis and that of the nasal schistosome are two different entities and naturally the adults that develop from these two should also be different. It is interesting to note that Sewell [1930] in his classification of the Apharyngeal Brevifureate Distome Cercariae, places Cercariae Indicae XXX in his Group I, along with S. haematobium, S. japonicum and S. mansoni, in which the cercariae have three pairs of flame cells in the body and one pair in the tail. In his Group II, which shows the first step in evolution in the flame cell pattern, he places the cercariae of S. spindulis in which the posterior flame cell in the body is an additional one. Hence there are four pairs of flame cells in the body and one pair in the tail in this group of cercariae.

The author infected over 40 clean Limnea leuteola (Lamarck) and Planorbis exustus (Deshayes) with miracidia of nasal schistosomes from experimental calves in April 1933. The mortality in among those snails was high and only about six Limnea and 14 Planorbis survived at the end of seven weeks and of these two Limnea and one Planorbis were found to discharge Cercariae Indicae XXX. This is another piece of confirmatory evidence in the life-history of the Nasal Schistosome. A section of the digestive gland of one of the infected snails is seen in Pl. I, fig. 4.

#### EXPERIMENTAL INFECTION AND INCUBATION PERIOD.

The cereariae chosen for infection were concentrated and enumerated according to the method adopted by Fairley and Mackie [1930]. Of the three calves selected for infection, calf No. 410 was drenched with 60,000 cercariae, calf No. 411 was infected through the mucous membrane of the nose with 60,000 cercariae, and the third. No. 409, was infected with 15,000 cercariae through the nasal mucous membrane and 30,000 cereariae by drenching. Rao [1933] has already published a preliminary report on this experimental infection and it is enough to say here that the calf No. 411 showed typical ova and live miracidia in its nasal discharge in 84 days after the first date of infection, calf No. 409 showed them in 88 days and calf No. 410 took 100 days to show them in its nasal discharge. Hence the period of incubation appears to be about three months. This finding in experimental calves agrees with what Rao [1932] said after analysing the histories of naturally infected animals in Nammiandal village near Tiruvannamalai. It is seen that the calf drenched with cercariae took a longer time to develop the disease and at the same time the attack was a mild one. The other two calves, on the other hand, showed severe symptoms of the disease though calf No. 409 got a smaller dose.

In another set of experiments two calves were infected with large numbers of cercariae of S. spindolis. One calf No. 458 was infected only through the nasal

mucous membrane and another calf No. 459 was infected by drenching alone. In these two calves, the former showed plenty of ova in its dysenteric motions on the 46th day after the first date of infection and the latter showed a few ova on the 76th day. This experiment also indicates that the calf drenched with cereariae took a longer time to show the infection. It would appear from this that calves infected by drenching get a less intensive infection than those infected through a mucous membrane. It suggests, therefore, that the cercariae poured into the rumen get mixed up with the ingesta and most of them die there and only a few that come in close contact with the mucous membrane burrow through and get the chance to Fairley and Jesudasan [ 1930 ] infected two goats complete their development. by applying cercariae of S. spindalis to the tongue and mucous membrane of the mouth, while in another goat they poured the cereariae into the rumen through a stomach tube. They found, on post-mortem examination, a larger number of schistosomes in the goats infected through the mucous membrane, than in the one infected through the stomach. The authors themselves appear to be doubtful about the cercarial invasion, in situ, in the rumen and say that only those cercariae that happened to be regurgitated into the gullet and the mouth apparently completed their development into adults. The findings of the author in his experimental calves confirm this.

Therefore in nature, cattle, unlike buffaloes, apparently get infected through the mucous membrane of the mouth and gullet and possibly through the skin of the legs when they drink or wade through cerearial infected water. Presuming that the natural infection is by ingestion it would appear that continued infection daily over a considerable period is required, to produce such a degree of infection as could be detected clinically.

# EARLY SYMPTOMS IN EXPERIMENTAL CALVES INFECTED WITH CERGARIAE INDICAE XXX.

The first symptom seen in these calves was a redness of the masal mucous membrane, coryza and sneezing. These symptoms appeared in exactly six weeks after infection. About ten days later the mucous discharge increased and simultaneously the mucous membrane became oedematous, which caused the narrowness of the nasal calibre, giving rise to a peculiar blowing sound during respiration. About eleven weeks after infection, there appeared on the oedematous mucous membrane, raised patches studded over with tiny abscesses shotty to the touch. The contents of these abscesses showed typical ova of nasal Schistosomes. At this stage the nasal discharge became thickened and appeared more or less mucopurulent and later became rusty and showed streaks of blood. One interesting feature observed in calf No. 411 was a relief in symptoms of distressed breathing periodically. The

distressed breathing synchronised with the appearance of the raised patches with the shotty abscesses on its surface and when these abscesses burst, the patches seemed to subside somewhat and the breathing became less noisy. This confirms Malkani's [1933] observations, apparently in advanced cases, in which the growths are said to undergo disintegration and get sneezed out leaving a raw surface and when this occurred the animal was temporarily relieved of the distressed breathing till the lesions increased in size again. In the experimental calves Nos. 400 and 411, the breathing became so difficult, during certain periods, as to interfere with feeding and rumination, with the result that they fell off condition. Experimental calf No. 410, which was infected by drenching with cercariae showed only a few discrete abscesses on slightly tunnified nucous membrane, and beyond slight blowing during respiration, did not "snore" while breathing as the other two calves did. A sagittal section of the nasal chambers of this calf is shown in Pl. II, fig. 1.

# POST MORTEM FINDINGS.

Calves Nos. 409 and 410 were destroyed, but calf No. 411 died after an injection of Tartar Emetic. Sagittal section of the heads of these calves were examined. Raised patches with abscesses and ulcers were found mostly in the vestibule of the nasal fossae, on the lower half of the turbinated bones and on the walls adjoining. In calf No. 411, a few discrete abscesses were also found high up on the turbinated bones. In all these cases, the veins on the floor of the nasal cavity and on the dorsal, ventral and alar folds of the nasal mucous membrane, looked corded and prominent. This is of some significance, because, these branches of the Sphenopalatine vein seem to form plexuses in those places and in them were found a large number of schistosomes. When the epithelium of this part of the mucous membrane was stripped, the schistosomes in the veins underneath were made visible and collected with ease. In fact from calf No 409, in about an inch and a half square of the mucous membrane from these regions, over 40 pairs and 10 single schistosomes were collected. It has been possible to collect over 300 schistosomes from portions of nasal lesions of the three experimental calves.

From the situation of the nasal lesions, in the early cases, it would appear that the lesions begin in the folds of the mucous membrane referred to and the floor of the nose just below the turbinated bones and from here they extend superiorly up to the lower part of the olfactary region as the cases became chronic.

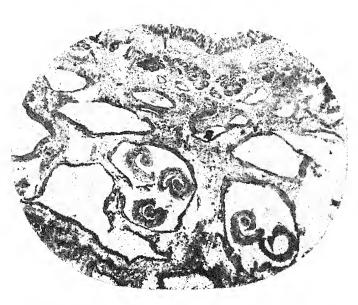
Soon after the calves were destroyed, the schistosomes were collected and they were all alive. It was therefore possible to study them alive and also to note any variation in their lengths caused by different methods of fixing, etc.





1. Section of head; calf 410 showing lesions.

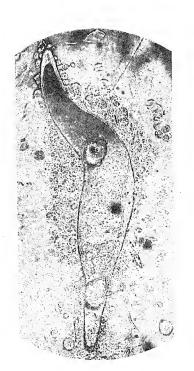
2. Note the proximity of worms to the surface. Note the number of veins.



3. Submucosa contains large veins, some big enough for three pairs.

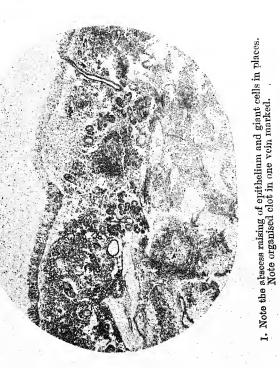


3. Proliferation of the epithelium into the tissue.



2. Note the ulcer reaching the vein containing worms.

4. Ovum of S. nasalis. Note the bubbles inside the horns of the ovum.



The mucous membrane of the maxillary and frontal sinuses was found to be in a state of inflammation in patches but the scrapings of these failed to show any ova of schistosomes.

In calf No. 411 which died 38 hours after an injection of 5 grains of antimony Tartaratum in 20 c.c. of water, an interesting and what appears to be an important finding, was a large number of nasal schistosomes in the clot of blood in the right heart, pulmonary artery and its branches. This calf died suddenly, apparently of heart failure, due to the dead schistosomes finding their way in large numbers into the jugular vein and then to the heart and blocking the branches of the pulmonary artery. Apparently the death of the calf was not due to the toxic action of the drug, 5 grains to 200 lbs. of body weight. From a portion of the nasal lesions of this calf over 100 dead worms, mostly males, were collected. The lesions in the nose in this calf were much more pronounced than in the other two.

From the portal veins of calf No. 409, 416 male and 16 female S. spindalis were collected and form the mesenteric veins 60 males and 15 females were obtained. Calf No. 411 contained only a dozen pairs of S. spindalis in its mesenteric veins and 5 male worms in the portal vein. Calf No. 410 yielded only four males from its portal vein and 50 pairs from the mesenteric veins.

#### Some observations on histopathology.

Sections of the mucous membrane from various parts of the nasal fosse were cut and examined. It appeared from these that the important portions concerned are the turbinate folds of the mucous membrane, viz., the dorsal and ventral turbinate folds and the alar fold. In addition to these the mucous membrane of the lower parts of the turbinate bones and the lower parts of the nasal septum were also found involved. The mucous membrane of the vestibule has a covering of stratified squamous epithelium and the rest of the region above is covered over with pseudostratified ciliated epithelium. The underlying mucosa contains a line of tubuloalveolar glands having mucous and serous alveoli and in between these glands there are many small capillaries. Below the line of these mucous glands, the sub-mucosa shows, particularly in these turbinate folds, a rich venous plexus, simulating cavernous tissue having several strata of freely anastomosing veins. Sections from an early case of nasal schistosomiasis show in these areas, numerous schistosomes inside almost all the veins adjoining the line of mucous glands. [ Figs. 2 & 3, Plate II & Fig. 2, Plate III.) It is seen that the ova which are so near the surface are liberated by way of the miliary abscesses and are thrown out to complete their biological cycle. Sometimes the ova get into the glands which get destroyed owing to the abscess formation. These early miliary abscesses just fill the thin strip of

tissue above the plexus and raise the epithelium which ultimately gives way, allowing the ova to escape. (Fig. 1, Plate III). Hence the time taken by these abscesses to burst is less in the early phases of the disease and naturally therefore tissue reaction is also less than in older cases. In these early abscesses there is evidence of the formation of a few plasmodial giant cells around some ova and only around these ova there appears to be a very thin layer of "actino-body". The presence of typical "actino-body" on the egg shell is apparently found on those ova which have to migrate from a deeper layer of tissue formed in lesions of some duration. It is interesting to note here that Hoeppli [1932] remarks, that the radiating structures observed on the shell of the eggs of S. japonicum, in sections of tissue, represent the secretion of the lateral glands of the miracidium which becomes strongly oxyphilic outside the egg shell and the effect of the secretion is probably one of softening and destruction. The latter portion of his observations confirms what Rao [1932] said about the formation of the "actino-body". In sections of nasal lesions from cases of just over two months duration many typical pseudotubercles with zones of young fibrous tissue of varying thickness are met with. Since these abscesses are formed repeatedly in the layer of tissue containing the glands, plenty of granulation tissue is formed and added repeatedly, causing an increase in thickness of the tissue there and due to uneven contraction of this fibrous tissue undulations appear on the surface of the mucous membrane. The size of these undulations depends on the severity of the contractions and the duration of the disease. The undulations are covered over with the tumified mucous membrane studded with abscesses, and in long standing cases such undulations represent the so-called sessile nasal growths.

As regards the blood vessels, they are all in a state of complete or partial engorgement. The bilharzial toxin present seems to elicit demonstrable cellular response. There is perivascular infiltration and also intercellular oedema in the surrounding tissue. Schistosomes are found free in the veins near the line of mucous glands and even in deeper strata. Some of the veins are almost exposed after bursting of these abscesses and if, owing to pressure, the vein ruptures there is haemorrhage and also escape of the worms with the blood. (Fig. 2, Plate III.) In some veins, clots are found surrounding dead schistosomes apparently undergoing phagocytosis in situ. The walls of such vessels are in a state of disintegration. In some veins the blood clots are in a state of organisation with or without canalisation. In calf No. 410, there appeared to be complete obliteration of the larger blood vessels and the whole area was a mass of fibrous tissue.

The size of the blood vessels in these folds of mucous membrane and in the base of the nasal septum is apparently large enough for even two or three pairs of worms together. (Fig. 3, Plate II.) It appears therefore that the veins in the

plexus in those places permit enough expansion for easy lodgment of schistosomes which enable their full development.

In sections of almost all growths from the nose from animals, even from those cases of only two months duration, it was always possible to see the epithelium growing into the tissue especially in places where frequent bursting of abscess leave behind ulcers. In places it appeared as if the epithelium was infiltrating into the tissue. This proliferation of epithelium was well seen in the vestibule where the mucous membrane is covered by stratified squamous epithelium. (Pl. III, Fig. 3.) Some of the mucous glands show increased secretion in their acini and ducts. In some places the epithelium of the ducts of these glands show proliferation. In some ducts, ova are occasionally met with acting as so many plugs. The mucous glands in many places showed degeneration. Fairley and Mackie [1930] suggest that there is a tendency to hepatic carcinoma in S. spindalis infection and in the lesions of the nose there appears to be a similar tendency of epithelial proliferation. Dew [1923] also mentions the epithelial proliferation in the bladder caused by the frequent escape of schistosome ova.

An interesting feature is that the bilharzial pigment is almost absent in the lesions of the nose when compared to the quantity present in the lungs of these cases.

The sections of lungs from ealf No. 411 showed that all the blood vessels were engorged with exudation in the alveoli in some places, causing a picture akin to venous congestion. The amount of pigment present in the lungs was very great, and most of it was in the endothelial cells of the capillaries. In some of the capillaries the pigment was seen in broken chains in the blood. (Pl. V, figs. 2 & 3.) The sections of lungs from calves Nos. 409 and 410 showed evidence of what looked like worm embolism in the smaller branches of the arteries with the usual tissue reaction and changes near it. A few pseudo-tubercles were also present. (Pl. V, fig. 4.)

#### EXPERIMENTAL INFECTION WITH CERCARIAE OF S. SPINDALIS

After the publication of the paper on the identity of the nasal schistosome by Bhalerao [1932] these experiments were done to find out if S. spindalis produced nasal schistosomiasis. The finding of S. spindalis in the experimental calves infected with nasal schistosomiasis was an expected contingency since the snails collected at Bandarlapalli harboured both the cercariae of S. spindalis and Cercariae Indicae XXX, the larval nasal schistosomes. Though there appeared to be no snail showing double infection, yet it was possible to have overlooked a couple of snails discharging cercariae of S. spindalis which got mixed up with the others. Two calves were chosen for infection and the cercariae of S. spindalis were obtained

from snails collected from Spur Tank, Egmore, a locality which is apparently free from nasal schistosomiasis. Great care was taken to examine large numbers of cercariae from each snail before they were utilised for experimental infection. Calf No. 458 was infected through the nasal mucous membrane and it received a total of 127,000 cercariae in 14 days and calf No. 459 which was drenched with an equal dose of cercariae over the same period. It is interesting to note that call No. 458 passed blood-stained facces on the 40th day and from the 46th day onwards its facces showed a fairly large number of typical ova of S. spindalis. This animal did not show any lesions nor ova of schistosomes in its nasal discharge and it was destroyed on the 86th day after infection. If S. spindalis were the cause of nasal granuloma, with the enormous dose of cercariae of S. spindalis which this animal received in comparison to the dose of Cercaria Indicae XXX received by calves Nos. 411 and 409, the calf in question should have developed the lesions in the nose within the same time as taken by calf No. 411. Calf No. 459 was kept under observation for 118 days and likewise neither showed any nasal lesions nor any ova in the nasal discharge as compared with calf No. 410 which did so in 100 days after drenching with a smaller dose of Cereariae Indicae XXX. These findings are, in the author's opinion, very significant. In calf No. 459 ova of S. spindalis could be detected in the facees in small numbers from the 70th day onwards, and it passed blood stained facces occasionally.

#### POST-MORTEM FINDINGS IN CALVES INFECTED WITH S. SPINDALIS.

The nasal cavities in both the calves were examined and no lesions were present. The scrapings from the nasal mucous membrane did not reveal any ova of schistosomes. The portal system of calf No. 458 was teeming with S. spindalis. From this calf over 4,000 worms, including females, were collected and as many worms perhaps remained uncollected in the smaller radicles of the mesenteric and the portal veins. In all the females examined, there were a large number of ova in the uterus of each and each ovum possessed the typical shape obtained for that species. (Pl. I, Fig. 3.)

The worms from the portal vein were all counted and there were 916 single males and 57 pairs. The percentage of males was about 94.1 and that of the females 5.9. Of the parasites collected from the mesenteric veins about 96 per cent. were pairs and 4 per cent. were single males.

From calf No. 459 only one dozen single males and two pairs were collected from the portal vein and over 250 pairs were removed from the mesenteric veins. The percentage of males to females in the portal vein was 83.3:16.7 respectively.

Sections of the liver, large and small intestines from these calves, particularly from calf No. 458 revealed a very large number of pseudo-tubercles and verminous

phlebitis. It was also seen that many of the ova in the liver and the intestines had "actino-bodies" on them. (Pl. IV, figs. 1 & 2.) The thickness of the "actino-body" and the number of ova that showed it appeared to be less than that found in nasal schistosomiasis. The pathological changes brought about in these organs seem to justify the belief that S. spindelis is likely to undermine the health of the animal. There is reason to believe that some cattle die of portal and intestinal bilharziasis and apparently the disease is not recognised as such. It is contemplated to deal with this question in another paper.

It is interesting to note that the sections of lungs from these two calves infected with S. spindalis did not reveal any schistosomes or their ova in the blood vessels, though a small quantity of bilharzial pigment was seen distributed here and there in the capillaries. A fairly large amount of pigment was seen particularly in the liver along the course of the sinusoids.

SOME OBSERVATIONS ON THE SCHISTOSOMES COLLECTED FROM ANIMALS IN THE ABATTOIRS.

The author had opportunities of studying schistosomes collected from sheep in Bellary and Anantapur Districts where he found mostly S. indicum and in a few cases S. spindalis as well. In Anantapur District he noted deaths in sheep due to diarrhoea, and in the faeces of these animals ova of these schistosomes were often found, showing that both species lived in the same animal.

During December 1932 over 112 bovines and 170 sheep and goats were examined for schistosomes in the Abattoir at Madras. None of the animals examined had nasal granuloma, and from 4 bovines and one sheep over 500 schistosomes were collected. In the collection from the portal veins it was found that the proportion of males to females was 16:1. In one bovine and one sheep both S. indicum and S. spindalis were found, and the identification of these was made by noting the shape of the ova in the available pairs with females having ova in utero. In this collection none of the parasites had the characteristically large tuberculations found in the nasal schistosome, nor did any females show ova typical of that species. The male S. spindalis appeared to possess smooth or slightly roughened cuticle and the male S. indicum showed only moderately developed tubercles.

Fairley and Mackie [1930], found that the sexes of all the worms collected by them, from eleven experimental goats, showed that the males were more than twice as numerous as the females. The findings of the present writer are in agreement with their observations.

THE HABITAT OF S. SPINYDLIS AND THE NASAL SCHISTOSOME.

Montgomery [1906], Liston and Soparkar [1918], Fairley and Mackie [1930] and the author have found that S. spindalis inhabits the portal and mesenteric

veins. Fairley and Mackie found in goats, infected heavily with cercariae of S. spindalis, an overflow distribution of schistosomes in atypical situations in the early phases of the disease, and later a biological equilibrium became established between the worms and the definitive hosts, when the worms limit themselves to their ordinary habitat, viz., the portal and mesenteric veins. They also say that the distribution of S. spindalis, within the blood vascular system, corresponds to S. mansoni and S. japonicum than to S. haematobium. The experimental infection in calves here, with massive doses of cercariae of S. spindalis, failed to produce nasal schistosomiasis, in other words, it means that S. spindalis does not inhabit and develop in the nasal veins, and the adult worms are found only in the portal and mesenteric veins, consequently the ova are voided only with the faeces of the host. As regards the nasal schistosome, the author has not been able to obtain any in the portal or mesenteric veins of animals examined in the abattoir or in the calves experimentally infected with nasal schistosomiasis. The absence of these in the portal system and the absence of their ova in the faeces or urine of the animals having nasal schistosomiasis at once suggests that these parasites do not develop in the portal or mesenteric veins. The ova of S. spindalis, on the other hand, have not been met with in the nasal discharge. It would therefore appear that the nasal schistosomes choose the nasal veins only as their habitat and develop there, and S. spindalis inhabits only the portal and mesenteric veins.

It would be interesting to note here the findings in two guinea-pigs, one infected with Cercariae Indicae XXX and the other with Cercariae of S. spindalis. In the former a few schistosomes were found in the sections of the lungs only and all of them appeared to be males. In some places two worms were met with, the side of one fitting in the gynecophoral canal of the other. (Fig. 4, Plate IV, & Fig. 1, Plate V). The latter guinea-pig showed only a few male S. spindalis in the portal vein and none in the lungs. One white rat was infected with Cercariae Indicae XXX, it was destroyed after eleven weeks and it failed to show infection. It is interesting to note that the guinea-pigs showed only male schistosomes developing in them, though they were infected with portions of the same material used for infecting the experimental calves in which both males and females developed. It cannot therefore be considered that the cercariae used for infecting these small animals may have been unisexual. This supports the suggestion of Fairley and Mackie [1930], that some host factor in the guinea-pig, antagonistic to the development of the female schistosomes, underlies the phenomenon of exclusively male survival, rather than initial unisexual infestation. Though these guinea-pigs had only male worms developing in them, yet the usual changes were found in the lungs of the one infected with cercariae of nasal schistosome and in the liver of the other infected with cercariae of S. spindalis.



Fig. 1. Section of liver showing "actino-body" round ovum of S. spindalis.

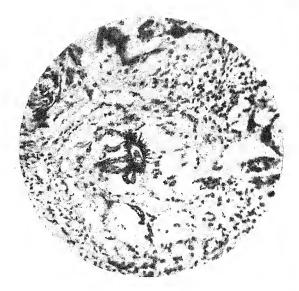


Fig. 2. Section of intestine showing "actino-body" round ovum of S. spindalis.



Fig. 3. Posterior end of male S. nasalis showing anastomosis and diverticulae of the common caecum.

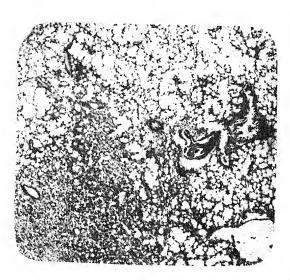


Fig. 4. Section of lung of guinea-pig showing S. nasalis and changes in the lung.

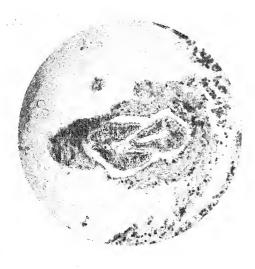


Fig. 1. Section of the lung of guinea-pig. Note two males interlocked.

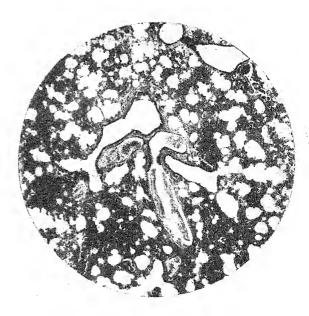


Fig. 2. Section of lung of calf 411. Note section of worms and changes in lung.

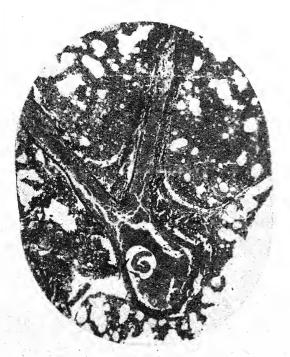


Fig. 3. Section of lung of calf 411. Note pigment in broken chains in the branches of the vein.

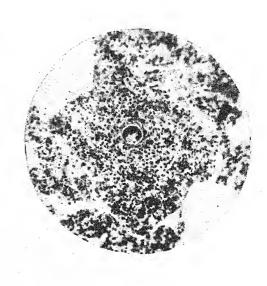


Fig. 4. Section of lung of calf 411. A forming pseudotubercle with ovum in centre.

Such pathological changes, due to the toxic effects of only male schistosomes, have been recorded in man and animals by Fairley [1924] and 1926], Fairley and Williams [1927], Faust [1927], Girges [1929], and Fairley and Mackie [1930]. The selection of the respiratory system by the nasal schistosome and the portal system by S. spindalis, even when reared in guinea-pigs, appears to be of some significance. Fairley, Mackie and Jesudasan [1930], found that when guinea-pigs were infected with S. spindalis the worms were confined to the portal system. The author's findings confirm this. Brumpt and Chevallier [1931], infected some mice with cercariae of S. mansoni and a few others with S. haematobium and found that in the latter the lungs were usually affected in addition to the liver, whereas in the former the reverse was the case. This supports the view that the nasal schistosome resembles S. haematobium in its behaviour in selecting its habitat.

DISCUSSION ON SOME POINTS OF DIFFERENCE IN THE MORPHOLOGY OF S. SPINDALIS AND THE NASAL SCHISTOSOME.

- (a) Size:—It has been admitted that the average size of schistosomes may vary with the age of the parasite, mode of preservation, time of collection after the death of the host, etc., but still the question remains whether or not there is difference in size between the different species. From the dimensions given in the text books for various species, it is agreed that different species have different sizes-a character which is useful when considered along with other morphological details. The dimensions of the nasal schistosome have been recorded by Bhalerao [1932] and Rao [1932] and from these it is gathered that this species is smaller than S. spindalis Montgomery 1906. Bhalerao, however, believes that the nasal schistosome fails to reach the maximum size attained by other schistosomes, because the former grows in a tough tissue like the nasal septum and does not get the same scope for development as those growing in the portal or mesenteric veins. This explanation seems to be untenable because the different species of schistosomes developing in the portal system of man and animals differ in their average sizes, even though they all have equal scope for development there. It has been shown above that the nasal schistosomes live in the veins of tissue, which resembles cavernous tissue, having several strata of freely anastomosing veins and in such tissue they necessarily get as much scope for development as the others have in the portal system. It would seem therefore that the site of infection has no bearing on the variation in size met with, apart from the character of the species.
- (b) Nature of the cuticle:—It has been said above (p. 19) that sheep harbour both S. spindalis and S. indicum at the same time and that only the latter showed moderately developed tubercles on its cuticle. An examination of the collection of schistosomes that exists in the Madras Veterinary College Museum

revealed only a large number of males disproportionate to the number of females, very often not showing any ova or only one species showing ova typical to that species, while the other species is immature. In such a collection, if it were a mixture of S. indicum and S. spindulis, and if one species were immature, one is apt to think that they are all one species because they are all from one animal and a few females showed only one kind of ova. Hence an observation on the nature of the cuticle in such a collection, may not be correct for obvious reasons. It has been noticed by the writer that the tendency of a person, when asked to collect schistosomes, is to do so from the portal vein only, unless he is particularly interested in the work, and naturally in a collection of that sort males predominate, consequently the identification often becomes a difficult matter, and may make one think that they are all one species.

It is interesting to note that Bhalcrao [1932] found variation in the development of tubercles on the cuticle of the schistosomes, which he believed to be S. spindalis, collected from a sheep, and this collection was from Madras, therefore it very likely contained S. indicum and S. spindalis, for reasons stated already. He also found that the cuticle of S. spindalis collected from the portal and mesenteric veins of two Bihar bulls having nasal schistosomiasis, was absolutely smooth. It was possible for the present author to examine the cuticle of over 2,000 males of S. spindulis obtained from the experimental calves and also from other animals and he found that the cuticle of the male in this species had no tubercles on it. If a variation in the nature of the cuticle did exist in S. spindalis it should have been evident during the examination of such a large number of parasites and its absence is significant. Fairley and Mackie [1930] in their studies in S. spindulis do not say anything about the nature of the cuticle, but from the excellent photographs which they gave of sections of these parasites in various organs, it seems that the cuticle of the male is non-tuberculate. On the cuticle of the nasal schistosome, on the other hand, large tubercles are seen, and even in the photographs of sections of these parasites in tissue, the large tubercles can be recognised.

As regards the nature of the cuticle of S. indicum and the nasal schistosome, the author agrees with Bhalcrao in considering that S. indicum has moderately developed tubercles and that the nasal schistosome has conspicuous and large tubercles on its cuticle.

Leroux [1933] states that the presence and degree of development of these cuticular bosses are greatly affected by the state of vitality of the specimens at the time of preservation. The large number of S. spindalis and nasal schistosomes examined by the present writer were reared, collected and preserved, under similar conditions, and no appreciable variation occurred in the nature of the cuticle.

Particularly in the nasal schistosomes the tuberculations were unaffected, even when they were collected from lesions sent in normal saline solution from the districts, and which arrived in the laboratory one or even two days after removal of the lesions. Therefore, it is evident that the tuberculate or non-tuberculate nature of the cuticle is of value when considered along with other morphological features.

(c) Number of testicular glands:—The number of testicular glands present in S. spindalis is 6 or 7 according to Montgomery [1906]. Bhalerao [1932], however, notes in his description of this parasite, that the number of testes varies from 3 to 6 and remarks that Montgomery might have failed to notice the range of variation in this respect. At the same time, he apparently ignores Montgomery's finding which, when taken into account, gives the range of variation from 3 to 7. The author examined over 300 male S. spindalis and found the range of variation to be 3 to 7 which is in conformity with the above. It is interesting to note that among those males 4 per cent. had three, 20 per cent. had four, 28 per cent. had five, 34 per cent. had six and 14 per cent. had seven testicular glands. Further the contour of each gland was inclined to be angular.

As regards the testicular glands in the nasal schistosome, the author examined 70 stained and over 40 fresh specimens of male nasal schistosomes from the masal lesions of the experimentally infected calves and it appeared to him that the range of variation was from 2 to 4 which confirms what he [Rao, 1932] found in the few specimens he collected from the lesions of animals in Nammiandal and Tiruvannamalai. Of these males 11 per cent. had four testes; 32 per cent. had three and 57 per cent. had four testicular glands. The contour of each testicular gland was found to be irregularly elliptical. If this is of any significance it indicates that the range of variation in testicular glands in the nasal schistosome is less than that obtained in S. spindalis. It is a fact that variation in the number of testicular glands is a character of the genus Schistosoma, but from a study of the variation in the number of testes in the well-known species it is evident that the variation is within certain limits for a given species. Hence this variation of testes is a factor in the determination of a species.

(d) The length of the common caecum in the female, no doubt, may appear to be variable in different individuals and consequently the situation of the ovary in the body of the female also will vary. Even then the maximum and minimum lengths of this structure compared with those of another species, gives some interesting information. According to Bhalerao [1932] the common caecum of S. spindalis measured 4·19 to 6·93 mm, and that of the nasal schistosome measured 4·28 to 5·23 mm. Montgomery [1906] said that the common caecum of S. spindalis was 6 mm. It would appear, therefore, that the maximum length of the caecum

of the female nasal schistosome is shorter comparatively than the other and this confirms Rao's finding.

It is interesting to note that one male nasal schistosome out of over 110 examined, showed branching and reuniting of the common caecum whereas such a thing was not seen in the specimens of S. spindalis examined. (Fig. 3, Plate IV.)

THE IDENTITY OF THE NASAL SCHISTOSOME.

In the foregoing pages the writer has endeavoured to establish that there is difference between the nasal schistosome and S. spindalis Montgomery 1906, in the

- 1. shape of the ova.
- 2. morphology of the miracidia.
- 3. morphology of the cercariae.
- 4. morphology of the parasites as regards their size, nature of their cuticle, the number of testicular glands they possess and the length of the common caecum of the female.
- 5. habitat of the worms.
- 6. choice of the intermediate host.

In addition to these differences, experimental evidence showed that infection with cereariae of S. spindalis failed to produce nasal schistosomiasis. Further it appeared to the author that the nasal schistosome is capable of affecting the respiratory system. It is therefore evident that the schistosome producing nasal schistosomiasis (Nasal Granuloma) in cattle is a new species to which Rao [1932] has already suggested the name "Schistosoma nasalis".

#### SPECIFIC DIAGNOSIS.

Schistosoma.-

Male.—Length 6.3 to 11 mm. Cuticle coarsely tuberculate. Testicular glands ranging from 2 to 4. Occasional branching and reuniting of the common caecum as in S. bovis.

Female.—Length 5 to 11 mm. Ovary post-equatorial. Uterus containing 1 to 5 ova. Length of common caecum about a third of that of the parasite. Ova spindle shaped, asymmetrical, having concavo-convex margins. Size 336 to  $581~\mu \times 60$  to  $80~\mu$ .

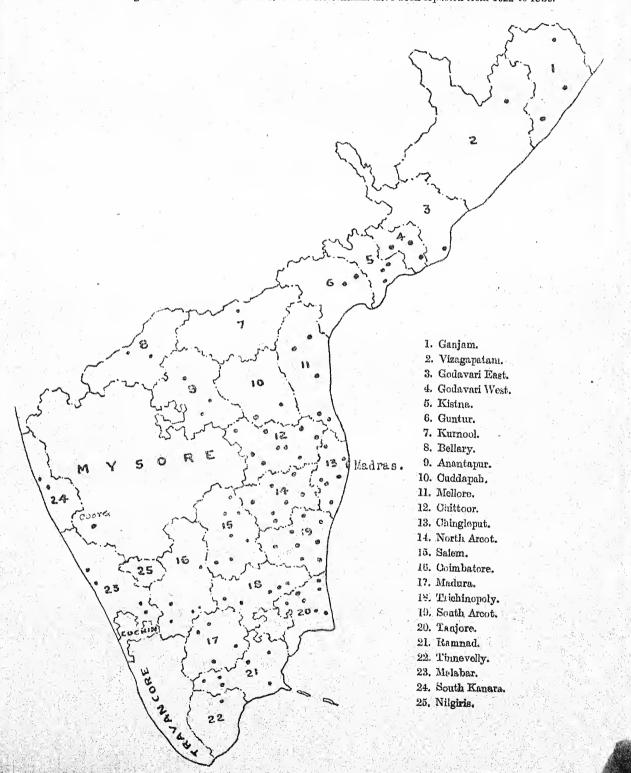
Hosts.—Cattle and buffaloes.

Location.—Veins in the turbinate folds of the mucous membrane of the nose. Locality.—India.

#### GENERAL REMARKS.

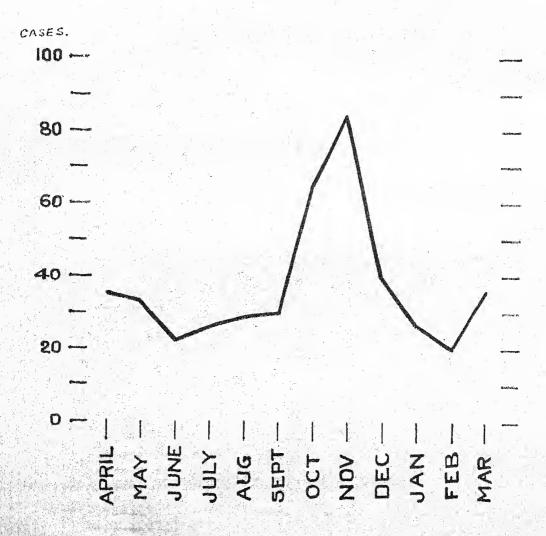
A map of the Madras Presidency showing areas from which cases of Nasal Schistosomiasis (Nasal Granuloma) were reported from 1922 to 1932 is appended

Fig. 4. Showing areas from which cases of Nasal Schistosomiasis have been reported from 1922 to 1932.



and also a graph showing the number of cases of Nasal Schistosomiasis reported from districts of the Madras Presidency based on the figures obtained from the Registers of the Laboratory from 1922 to 1932. From the spot map, it is evident that Nasal Schistosomiasis is widespread in this province, and the disease appears to be more prevalent in the "dry" districts, which depend on tanks and poinds for water supply to their cattle.

Fig. 5. Showing number of cases of Nasal Schistosomiasis reported in the Madras Presidency from 1922 to 1932.



Though the graph cannot be taken as one showing the seasonal incidence, because it is not based on the records of a chosen area, yet it does show that a large number of cases were recorded during the cooler season of the year. No work on the study of seasonal incidence has been done and if such a work is undertaken in a chosen area and simultaneously if the incidence of *Cercariae Indicae XXX* is also studied in the same area, very valuable information will become available which should be of much use in adopting ways and means of prophylaxis.

### ACKNOWLEDGMENTS.

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## SURRA IN THE PUNJAB.

BY

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The object of the present note is briefly to review the operations carried out by the Civil Veterinary Department in the Punjab, in connection with the treatment of Surra of equines and camels during the seven years ending with March 1933.

Surra in the Punjab recently assumed terrifying proportions, causing enormous economic losses in horses, camels, sometimes cattle and packs of hounds. In some villages lying in waterlogged areas, there was not a single equine remaining alive in 1926-27, and in 1925-27 a great number of cattle died.

It is not proposed to consider here the results of trials carried out with some of the earlier remedies, such as tartar emetic used alone or one or more of the several arsenical preparations recommended from time to time for the treatment of this condition. With the introduction of Naganol in the field of veterinary medicine, the records of treatment with these drugs have come to be regarded as so many "dead" chapters in the history of chemotherapeutic endeavours and it may almost be declared that the accumulated records of work carried out during the past decade in the treatment of Surra in various countries are essentially the records of the application of this new remedy.

The treatment of cattle and camels now presents no considerable difficulties, but there appears to be a certain diversity of opinion, even amongst laboratory workers, as to the method of treatment best calculated for obtaining cures in the case of equines. A very genuine difficulty therefore confronts the field veterinary worker who has largely to base his decision in this respect upon the results of work carried out in the laboratory. This does not affect Army horses only, but also those owned by zamindars and horse breeders, and to these classes they still represent a very important economic factor.

The two considerations which very largely weigh with the field worker in the choice of a remedy are its cheapness and practicability, but the importance of these is apt to be underrated by the laboratory worker with whom the sterilization of the circulating blood of the affected animal constitutes almost the sole object of his investigation.

Thus, while the combined intravenous and intrathecal treatment, as elaborated by Edwards [1928], must be regarded as having registered a definite advance in the chemotherapy of equine surra, its application under field conditions presents considerable difficulties, for it must be borne in mind that the staff at the District Veterinary hospitals consists normally of a Veterinary Assistant, a compounder, *bhisti* and sweeper, and sometimes the latter are part-time men. The intrathecal treatment necessitates the throwing of and carefully securing the animals, and when it is realised that at one centre (Muridke) there were four hundred animals undergoing treatment at one time, it will be understood that the intrathecal method is not practicable, and opens up the possibility of risks, which are not encountered in the intravenous method.

Again, in view of the high price of Naganol (about Re. 1 per gramme), it becomes a matter of immediate necessity to devise a method whereby it could be suitably combined with a less expensive drug, so as to bring about a substantial reduction in the ultimate cost of the treatment, and, as will be seen from what has to be stated presently. Surra in the Punjab is, at the present time, almost exclusively treated with a combination of Naganol and tartar emetic.

#### I. TREATMENT OF EQUINE SURRA.

Before dealing with the actual results obtained under this category, it is perhaps desirable that a few words should be said as to the factors, other than the question of cost, that have weighed with the Department in choosing the combined form of therapy, to which a reference has just been made.

In the Dutch Indies, which perhaps represent the only territory, outside the Indian Region, where field-scale experiments have been undertaken with a view to developing a practical and economical method of combating equine surra, the earlier trials in this connection were directed to obtaining cures by means of Naganol used alone [Baermann, 1922; Rodenwaldt and Douwes, 1922], but it was evidently not long before it was realized that a much superior form of remedy consisted in a combination of Naganol and tartar emetic, and from the reports that have been published during recent years by Bubberman and his collaborators [1925, 1931], one gathers that the treatment of equine surra in that country is, at the present time, exclusively carried out by means of the Naganol-emetic combination.

The same combination would appear to have been used successfully by Bakker [1925] in the treatment of equine surra in Padang Sidempoean.

A discussion of the merits of this form of treatment on theoretical grounds would involve an encroachment upon the preserves of "pure research" and would be outside the scope of a note of this kind which is designed primarily to deal with

the practical aspect of surra treatment. A reference may, however, be made to the marked efficacy of the Naganol emetic combination for *Trypanosoma congolense* infection which is not usually amenable to treatment with Naganol alone [Berg, 1925]. It is possible that the superiority of the combined drugs over either of these used alone is due to the fact that, as suggested by Collier [1926], a "globulin-Bayer" compound is formed when Naganol is introduced into the circulating blood and that the interaction between this and the tartar emetic results in the formation of a new trypanocidal agent.

At the present time, the routine method of treating equine surra in the Punjab consists of intravenous injections at intervals of four or five days, in the following approximate dosage:—

1 per cent. solution of Naganol .	•			- 4	100 c. c.	
1 per cent. solution of tartar emetic	•	•	. • .	•	75	
1.5 per cent. solution of tartar emetic			, o •		33	
1.5 per cent. solution of Naganol .			•	•	,,	and the second
2 per cent. solution of tartar emetic				1	· ·	
$2^{\star}5$ per cent. solution of tartar emetic	•		•	- *		
2 per cent, solution of Naganol .		, (°•*	) • ·		"	

The increase in the dosage is not a fixed one, but depends upon the condition of the animal, and symptoms, if any, caused by the previous dose. A full course of treatment occupies approximately one month.

Up to the termination of March 1933, over a period of seven years, 5,692 equines had been admitted for treatment, 53 relapsed, and 485 died or were destroyed, while 78 were taken away before the completion of the treatment and 91 are untraceable or reported to have died, showing a percentage of cures of approximately 876.

The number which died under treatment includes those which were brought in a semi-paralytic condition, and emaciated animals, about which no hope of success was held out at the commencement. If these cases, and the ones which were removed before completion of the treatment, be excluded, the percentage of cures is approximately 97 per cent.

For the field worker, the development of the suitable method of treatment is not enough, inasmuch as the mere method would be valueless if means were not found to ensure its ready adoption by the populace. On account of former attempts at successful treatment having been somewhat abortive, it was difficult to induce the peasantry to feed their animals adequately while under treatment as experience had

shown them that the probability of receiving the animal back in a healthy condition was remote. On this account, the Punjab Government, at the instance of the Veterinary Department of the province undertook to feed all equines free of cost while undergoing treatment; this has cost the Government an increasing amount each year, the total from 1926 being about Rs. 37,000. The object of this suggestion was to demonstrate to owners that if the animals were well fed while undergoing treatment, the chances of recovery were very high, and it was decided that the feeding should continue for 4 or 5 years, after which the concession should be gradually withdrawn.

Until 1926, Surra treatment was to a great extent confined to the Sohawa laboratory, but on account of the present treatment, and the inability of zamindars to send their animals so great a distance, Surra centres attached to the hospitals were opened in suitable areas throughout the province. The Veterinary Assistants at these centres were specially trained for this work and the equines brought to these hospitals for treatment for surra were fed free of charge.

The success of this procedure has been phenomenal, and has resulted in the zamindars and breeders having great confidence in the hospitals and treatment. This is demonstrated by the fact that at one centre, Sheikhupura, the zamindars, instead of looking upon their animals as lost as in former times, are now buying better class horses and mares and have applied for a District Board stallion for breeding purposes. This instance is typical of others. The extent to which the confidence of the zamindar has been gained by the success of the treatment is illustrated by the numbers admitted for treatment yearly since the opening of the Surra centres:—

	1926-27	89 equines admitted.
i	1927-28	114 ,, ,,
	1928-29	109 , , ,
	1929-30	. 387
	1930-31	. 1,448 , ,
1	1931-32	. 1,965 ,, ,,
	1932-33	. 1,520

Whilst the results of the treatment itself have been very gratifying, more has been accomplished in that the owners have seen that under favourable conditions and given good food, their animals are almost certain of recovery provided they are placed under treatment reasonably early. This has resulted in a continually diminishing number being maintained by Government, and it is hoped that during the course of another year or two the burden of feeding them will be lifted, the only ones remaining being those of very poor owners and those brought from a long distance, which would preclude the possibility of repeated visits.

The success achieved by feeding the animals free of cost in order to persuade the populace to place their animals under treatment may be seen, in that during the year ending March, 1933, out of the total of 1,520 animals treated, more than a thousand have been fed at the owners' expenses, thus affording ample proof of the justification of Government's policy in incurring the expense of feeding the animals during the first few years.

During the early years of the treatment, when the number of animals to be treated was not so large, the infectivity of the blood of the treated animal used to be tested by subinoculation into a small animal, but now a postcard is given to the owner with a request that he should fill in the answers to the questions contained therein which refer to its condition, whether it is working or not, or whether it has succumbed, and return it after a period of three months. In addition, many thousands of pamphlets have been issued to zamindars in the region of the Surra centres requesting them to halt for a few minutes at the hospital, whenever in the vicinity, so that the blood of their animals may be examined. This procedure occupies only about five minutes of his time and has resulted in a great many animals being treated before the onset of the initial symptoms.

# II. TREATMENT OF SURRA OF CAMELS.

Except for the results reported from time to time by the Army Veterinary Department in India and by the Punjab Civil Veterinary Department, there is little information on record concerning the value of Naganol in the treatment of surra as it affects the camel. The drug would, however, appear to have been used with some success in at least two analogous conditions, namely, Su-auria and the form of camel trypanosomiasis caused by Trypanosoma soudanense, in the Anglo-Egyptian Sudan. In regard to the former, Ilowaisky and Zeiss [1923] claimed to have obtained cure in one case by a single injection of 3 grammes of the drug, although relapse occurred in another case treated in the same manner. In a later paper, Zeiss [1928], working in collaboration with Emelin, has declared that a single dose of 4 to 6 grammes of Naganol is usually sufficient for obtaining cures.

In regard to *T. soudanense* infection (which is believed by some to be identical with surra), the results obtained by Knowles [1927] in the treatment of this condition appeared to him to warrant the conclusion that "simple doses of less than 10 grammes may be sufficient to bring about a cure", although such doses "cannot be relied on to bring about complete destruction of the trypanosomes".

Quite recently, however, Bennett [1933], in discussing the efficacy of Naganol for T. soudanense infection, has expressed the opinion that "a single intravenous

injection of 4 grammes is sufficient". As to the previous occasional failures encountered in the treatment of this condition even when much larger doses of the drug were used, Bennett observes: "There is no doubt that in the earlier years of this drug's history it was, if not always, different from modern samples, at least more variable". Obviously, the author here refers to variability in the physical appearance of the samples, but it would seem open to question how far such variability might be held to account for any marked alteration in the curative properties of the compound.

In any case, judging by the results of work carried out in the Punjab, we cannot consider ourselves as having had sufficient evidence that treatment by a single injection of Naganol constitutes a cure for surra in camels, although the animals are maintained in work. This doubt is emphasised by the fact that the greatest number of relapses (20) occurred in the year 1931-32 when 140 camels were injected with a single dose of Naganol (4 grammes intravenously) as an experimental measure. The method of treatment adopted for camels has therefore been identical in form with that of horses, but the Government has not provided the feed for them. It is of interest in this connection that in the course of his early trials, Knowles (l. c.) had found that a single intravenous injection of 4 grammes of Naganol combined with 2 grammes of tartar emetic could effect a cure in T. soudanense infection in camels. The chances of recovery therefore seemed much greater with a course of treatment consisting of 4.5 grammes of Naganol combined with 7 grammes of tartar emetic (ante), and, as will be seen from the figures given below, the results obtained with this method of treatment have been very gratifying.

The numbers admitted for treatment from 1926 are as follows:-

	1926-27	-					V -			1.25	- "		- 89
	1927-28		1.0		F 1		1					-	142
	1928-29						+ 4						31
	1929-30				· 1	3.5				. 1			100
100	1930-31					- 1			• -				477
	1931-32		■4 3 × 1			- 1	-	1.7% •	<b></b>			3	676
	1932-33		#1871 Te	• .	11 200		4.			- N			620

Out of the total of 2,135 camels admitted for treatment during these seven years, 67 died or were destroyed, 33 relapsed, 179 were taken away before the completion of the treatment, and 3 are untraceable, or reported to have died, showing a percentage of cures of approximately 86.8. This percentage would be appreciably higher if one excluded the numbers of hopeless cases and those taken away before the completion of the treatment.

It may be argued that in the case of camels a month's treatment is unnecessary and cumbersome and that it is therefore desirable that further work should be undertaken with a view to exploring the possibilities of the single injection method of treatment with Naganol. This is possibly true, but experience has shown that in a large proportion of cases, a protracted treatment is definitely to the advantage of the animal concerned. This may seem paradoxical, but the truth of this remark will be apparent from the fact that most of the camels are brought for treatment in an emaciated condition, and even if unaffected by surra are certainly unfit for work. The month's treatment ensures that the animal enjoys a month's rest and has an opportunity of putting on a little condition.

In conclusion, it may be asserted that provided the animals are treated reasonably early in the course of the disease, and are properly fed during treatment, the intravenous method of treatment affords a certain cure for equines and camels, with the exception of a negligible number which may relapse two or even three times after treatment.

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# A STUDY OF THE DATA OF MILK YIELDS OF VARIOUS TYPES OF CATTLE OBTAINED FROM THE RECORDS OF GOVERNMENT MILITARY DAIRY FARMS.

I. RATE OF DECLINE IN MILK YIELD WITH ADVANCE IN LACTATION.

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(With seven charts)

## INTRODUCTION.

It is well known that the rate of milk secretion in dairy animals rises rapidly during the first few weeks following parturition, reaches a maximum and then declines more or less rapidly until the animal dries off. The length of the milking period depends largely on the service period, which is the length of the interval that elapses between calving and the recurrence of conception. If conception takes place within two to three months, the animal will remain in milk for about ten months and longer if conception is delayed.\* Of this milking period only during the first month is the yield found to be rising. The maximum is generally reached at the end of the first month, or in the second month, and the rest of the lactation period is associated with decline. The period of rise is thus very small compared to the period of decline; the yield for the lactation should, therefore, depend to a large extent on the rate at which the yield falls off. In the following pages it is proposed to examine this rate of decline in the case of herds of Indian and cross-bred cattle and buffaloes living under fairly good conditions of feeding and management obtainable in the Government Military Dairy Farms in India.

#### METHOD.

The rate of decline, or its complement, persistency, has been studied by various investigators in England and America, and various methods have been employed in

<sup>\*</sup> How far the lactation length depends upon service period will be seen from the correlation between the two. The coefficient of correlation is about 7. For every month's delay in service the lactation is prolonged on an average by a little over half a month.

measuring it. A review of the methods and the literature on the subject is beyond the scope of this paper. The term lactation curve is used for the curve that describes the rate of milk secretion with advance in lactation, and it has been shown that this curve is capable of simple mathematical expression. The form of the expression used here is the exponential equation\* of Brody and co-workers which has been successfully employed by Gaines and Davidson [1926] in their study of the rate of decline. Briefly stated, this consists in supposing that the decline takes place continuously at a constant rate proportional to the rate of yield at the time. The expression analyses the curve into the two well-known characters: the initial maximum rate of secretion and the rate at which it declines. The equation was first applied to the weekly yields.

#### DATA.

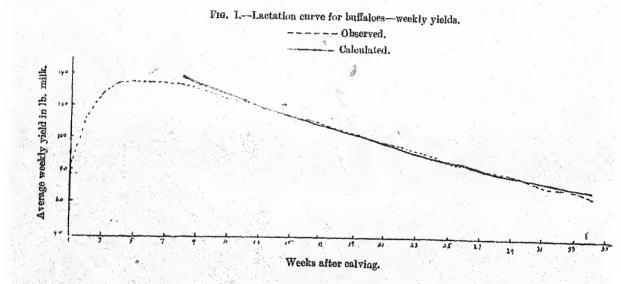
The data are obtained from the Milk Record Books of the Government Military Dairy Farms in India. These books contain the name of the animal, the dates of calving and of service, and the yields at each milking on all days from the date of calving to the date of drying. The daily yields were copied down on forms especially designed for the purpose. These forms contain the number and name of animal, the dates of calving and of service, service period and age. Seven-line columns are provided on the form with headings, first week, second week, etc., to represent all the weeks of the lactation. Often the date of service marked in the Record Books cannot be relied upon as it is not known whether that service has actually resulted in conception or not. Service periods were, therefore, obtained from the History Sheets of the animals by subtracting the gestation period (300 days in the case of buffaloes and 280 in cows) from the calving interval. Ages also were copied from the History Sheets. By totalling up each column of this form, weekly yields are obtained for all weeks from calving to drying.

<sup>\*</sup>As this is intended to be a popular article it is not proposed to weary the reader with the mathematics of the method. The equation made use of by Gaines and Davidson is  $dy/dt=ae^{-kt}$ , where y is yield in lb., t is time in months from calving, dy/dt is rate of yield in lb. per month, a is a constant representing theoretical initial rate of yield per month, and k is a constant representing the rate of change per month (as proportional to dy/dt) in the rate of yield per month. The k in this equation measures the rate of decline.

By persistency is meant the degree to which level of yield is maintained. Thus in terms of the above equation,  $100 \times e^{-k}$  is persistency, and  $100 \ (1-e^{-k})$  is the percentage monthly decline. As  $1-e^{-k}=k$  for small values of k,  $100 \times k$  is percentage monthly decline to a first approximation and  $100 \ (1-k)$  is persistency expressed as a percentage.

## RESULTS: BUFFALOES' WEEKLY YIELDS,

The data for buffaloes were first made use of for testing if the equation accurately represents them. There were 140 lactations obtained for buffaloes from the records of the Northern Circle. The average weekly yield of each week of lactation was worked out. These are represented by the dotted line in Figure I. It will be seen that the yield rises up to the 5th week, remains steady till the seventh and declines from the 8th. The equation was applied to the decline portion of the curve,



that is, to the data of yields from the 8th week of lactation, and the values of the constants, maximum and rate of decline were determined. The method of determining these is by what is known as method of least squares,\* the virtue of which is that the error is the least possible. The smooth curve obtained from the calculated data is represented by the continuous line in Figure I. From this it will be seen that the average buffalo starts with a maximum of 142.5 lb. per week in the 7th week of lactation and then declines weekly at the rate of .0265 lb. per lb. per week (which is about 2.65 per cent.). The observed and calculated values of the weekly yields are given in Table I. Column 5 of this Table shows by how much the

<sup>\*</sup> The method of calculation is explained in Appendix I.

TABLE I. Average weekly milk yield of buffaloes during weeks following maximum— All observations. (Maximum:—7th week.)

Weeks after	No. of records	Data of mill	yield in lb.		
calving	averaged	Observed	Calculated	Deviation	
8	135	134·3	138.8	-4.5	
9	136	131-2	135-2	-4.0	
10	* 139	127.9	131-6	3.7	
n	140	1260	128-1	2·1	
12	139	123.9	124.8	0.8	
13	138	120.8	121.5	-0.7	
14	139	117-7	1184	0.7	
15	138	115.5	115.3	0.2	
16	139	113.4	112.3	11	
17	140	110.5	1093	1.2	
18	139	108.4	106.4	2.0	
19	139	106.2	103.6	2.6	
20	139	102.4	100.9	1.5	
21	139	100.6	98-31	2.29	
22	138	98-8	95.72	3.08	
23	139	95.2	93.24	1.96	
24	138	92.3	90.70	1.60	
25	139	88.0	88-41	-0.41	
26	137	87.5	86.10	1.40	
27	136	85-5	83.85	1.65	
28	135	82.8	81.66	1.14	
29	133	80.4	79.50	0.90	
30 -	131	76.6	77.43	-0.83	
31	122	74.5	75.41	0.91	
32	116	73-2	73.43	-0.23	
33	109	69.5	71.52	-2-02	
34	100	66:6	69.63	-3.03	

Rate of decline per lb. per week:— 0265. Ratio of any week's yield to that of preceding week:— 9738.

calculated values deviate from the observed. The deviation is only about 1.6 per cent. of the average weekly yields. Such an error is not at all large for data like milk yields, which are in their very nature subject to very large variation, depending as they do on a large number of factors of heredity and environment. It will also be noted that the smooth curve cuts through the data admirably well.

## To compute yield for any period.

The milk producing capacity of an animal is thus described in terms of the two characters, the maximum and the rate of decline. The yield for a lactation or for any stated period can be calculated when these are known. Thus at maximum the buffalo yields 142.5 lb. a week. In the 8th week its yield would be diminished by 2.65 per cent., that is, the yield would be .9735×142.5 lb. In the 9th week the yield would be 97.35 per cent. of the yield in the 8th, i.e., .9735×.9735×142.5 lb.; in the 10th, .9735³×142.5 lb., and so on. By adding these quantities over the length of the lactation, or for any other period desired, the total yield is obtained. The calculation is laborious, no doubt, but with the aid of calculus it is very simple, the summation being performed by a simple process, namely, integration. Graphically also the yield for any period can be calculated without much labour. Yield is represented by area under the curve, and when the lactation curve is drawn, the yield between any two dates is obtained by erecting ordinates at these two dates and computing the area enclosed by them, which is done by counting the number of squares enclosed.

The data were next examined to see how the rate of decline is affected by pregnancy. For this purpose the lactations were sorted out into groups according to the lengths of service periods for the lactations. All calculations were made as before and the figures are given in Tables II to V.

TABLE II.

Average weekly milk yield of buffaloes during weeks following maximum—service period:—2—6 weeks. (Maximum:—5th week.)

	yields in lb.	Data of milk		
Deviatio	Calculated	Observed	No. of records averaged	Weeks after calving
—5·(	132·6	127·6	29	6 7
—4·1	128·8	124·4	28	
0·3	125·2	125•5	27	8
0·4	121·6	121•4	29	
-0.9	118·1	117·2	30	10
1.0	114·8	115·8	30	
0.9	111·5	112·4	29	12
	108·3	111·3	29	13
1.6	105·3	101.9	30	14
-0.4	102·3		30	15
0·1:	99·38	99·5	30	16
-1·0	96·56	95·5	30	17
-0.7(	93·8	93·1	29	18
1.1(	91·14	92·3	30	19
1.56	88·54	90·1	30	20
4.38	86·02	90·4		21
3·02	83·58	86·6	30	22
0·39	81·21	81·6		28
-1·20	78·89	77·6	30	24
1·05	76·65	77·7	29	25
0·03	74·47	74·5	29	26
0·06	72·36	72·3	28	27
0·70	70·30	71·0	27	28
1·91	68·29	70·2	26	29
-1·66	66·36 64·46	64·7 62·9	26 23	30
-3.43	62.63	59.2	21	32

Rate of decline per lb. per week :- 02885.

Ratio of any week's yield to that of preceding week: -- . 97 14.

42 THE INDIAN JOURNAL OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY [ IV, 1. TABLE III.

Average weekly milk yield of buffaloes during weeks following maximum—service period:—6—10 weeks. (Maximum:—7th week.)

			Data of mill	yields in lb.	
	Weeks after ealving	No. of records averaged	Observed	Calculated	Deviation
	deligacing and the state of the consequence of the state				
	8	43 43	130·6 128·1	135·8 132·0	-5·2 -3·9
	10	43 43	124·7 121·7	128·4 124·8	3·7 3·1
	12	43	119·3	121·4	-2·1
	13	43	115·7	118·8	-3·7
	14	43	112·1	114·8	-2·7
	15	43	111·8	111·6	0·2
	16	43	110·4	108·5	1·9
	17	43	107·8	105·5	2·3
	18	43	105·7	102·7	3·0
	19	43	103·8	99·79	4·01
	20	43	99:3	97·05	2·25
	21	43	96:8	94·36	2·44
	22	42	97·7	91·75	5·95
	23	43	92·7	89·23	3·47
	24	42	90·7	86·76	3-94
	25	43	84·02	84·37	0-35
	26	42	84·2	82:04	2·16
	27	42	82·4	79:77	2·63
	28	42	80·5	77·57	2·93
	29	41	74·5	7 <b>5</b> ·43	—0·93
	30	40	71·3	73·35	-2·05
	31	36	69·0	71·32	-2·32
	32	32	69·95	69·34	0·61
	33	30	64·6	67·44	-2·84
भ वर्ष चार्	34	25	59.2	65:57	-6.37

Rate of decline per lb. per week :- 02800.

Ratio of any week's yield to that of preceding week :- 9724.

TABLE IV.

Average weekly milk yield of buffaloes during weeks following maximum—Service period 10—14 weeks (Maximum—7th week).

Weeks after	1		lk yields in 1b.		
oarving	averaged	Observed	Calculated	Deviation	
8	22	136·97	143·1	-6·13	
	22	135·9	139·4	-3·5	
10	22	134·4	135·9	-1·5	
11	22	132·0	132·5	-0·5	
12	22	129·5	129·1	0·4	
13	22	127·2	125·9	1·3	
14	22	122·3	122·7	-0·4	
15	22	117·1	119·6	-2·5	
16	22	115·5	116·6	-1·3	
17	22	112·3	113·6		
18	22	113·3	110·8	2·5	
19	22	110·1	108·0	2·1	
20	22	105·7	105·2	0.5	
21	22	103·8	102·6	1.2	
22	21	102·9	100 0	2·9	
23	21	101·0	97·47	3·53	
24	21	90.8	95*01	4·09	
25	22		92·62	—1·82	
26	21	93·8	90·31	3·49	
27	21	92·3	88·00	4·30	
28 29 30	21 21	84·4 87·3	85·76 83·60	1·36 3·70	
31 32	20	84·9	81°49	3.41	
	19	79·7	79°43	0.27	
32 33 34	19	78·6 67· <b>5</b>	77·43 7 <b>5</b> ·48	1·17 —7·98	
35	17	70·5	73·57	-3.07	
36		71·2	71·71	-0.51	
30	13	67.5	69-90	-240	

Rate of decline per lb. per week-02558.

Ratio of any week's yield to that of preceding week-9748,

44 THE INDIAN JOURNAL OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY [ IV,  $\tau_{\star}$  Table V.

Average weekly milk yield of buffaloes during weeks following maximum—Service period 14—26 weeks (Maximum—7th week).

Weeks a	fter	No. of records	Data of milk	yields in lb.	
calving	g	averaged	Observed	Calculated	Deviation
8		30	139*7	143·5	-3*8
9		29	135*9	140·3	-4*4
10		30	133*7	137·3	-3*5
11	***	31	132·3	134·2	-1.9
12		31	130·1	131·2	-1.1
13		31	128·6	128·2	0.4
14		31	127·8	125·4	2·4
15		30	126·9	122·7	4·2
16		31	123·7	119·9	3·8
17		31	121·5	117·5	4·2
18		31	118·3	114·7	3·6
19		31	114·7	112·1	2·6
20		31	110·4	109·6	0·8
21		31	109·8	107·3	2·5
22		31	106·5	104·8	1·7
23		31	103·5	102·5	1·0
24		31	99·0	100·0	—1·0
25		31	96·5	98·04	—1·54
26		31	96:0	95·88	0·12
27		31	93:6	93·76	-0·16
28		31	91:2	91·68	-0·48
29		31	98.0	89·64	-1.64
30		31	84.7	87·66	-2.96
31		30	83.9	85·70	-1.80
32		30	81·2	83-83	-2·63
33		28	79·9	81-96	-2·06
34		27	79·7	80-15	-0·45
35		27	74·3	78·38	-4.05
36		24	75·5	76·63	-1.13
37		21	74·0	74·94	-0.94
38		20	78·0	73·28	4.72
39		19	75·0	71·66	3.34
40		18	71·2	70·06	1.14

Rate of decline per lb. per week-02239.

Ratio of any week's yield to that of preceding week-9782,

Although the population becomes very small when divided into the various service period groups, the error is never more than three per cent. of the average weekly yields. It will be seen that the rate of decline is affected but slightly by pregnancy. But no conclusion can be drawn from results based on such a small number. The subject will be examined in greater detail in a separate paper.

## RESULTS -ALL BREEDS.

It will be seen from the above that the equation made use of represents the data fairly accurately as far as the decline portion of the curve is concerned. But by representing the data by this equation five to seven weeks' yields have to be emitted. By taking monthly instead of weekly yields the equation may be made to represent a larger part of the lactation. Further, examination of more data showed that the variation from week to week is not so regular or marked; and this short-time variation also could be eliminated by taking monthly yields. The work was done, therefore, in the case of Indian and cross-bred cows with monthly instead of weekly yields, and for uniformity the same was repeated with buffaloes. A month was taken to be of four weeks. The first fortnight was omitted and average monthly yields were obtained, in the case of Indian and cross-bred cows and buffaloes, for full months beginning with the third week of lactation. The maximum and the rate of decline were obtained as before. The data are given in Tables VI to IX

TABLE VI.

Average monthly rate of milk secretion of cross-bred cattle, with advance in lactation.

Month after	No. of records	Milk yi	ield in lbs.	
calvirg	averaged	Observed	Calculated	Deviation .
1 2 3 4 5 6 7 8 9	157 157 157 157 157 157 157 157	946-9 902-9 834-7 754-5 679-9 617-5 565-3 515-0 462-7	980·9 894·5 816·8 744·0 678·5 618·9 564·4 514·7 469·5	-34·0 8·4 18·9 10·5 1·4 -1·4 -9 -3 -6·8

Rate of decline per lb. per month:—09215.

Ratio of any month's yield to that of preceding month:—9121.

Table VII.

Average monthly rate of milk secretion of pedigree Sahiwals with advance in lactation.

Month after	No. of records	Milk yield	* 11	
calving	averaged	Observed	Calculated	Deviation
1	112	779-5	791·1	-11.6
2	112	750-0	734·1	15.9
3	112	692.0	681.6	10.4
4	112	628.5	632.5	-4.0
5	112	585-0	587.2	-2.2
6	112	521.0	545.0	-24.0
7	102	518-5	505-9	12.6
8	102	<b>473</b> ·5	469.6	3.9

Rate of decline per lb. per month: - '0745.

Ratio of any month's yield to that of preceding month: - '9277.

Table VIII.

Average monthly rate of milk secretion of ordinary Sahiwal cows, with advance in luctation.

Month after calving	No. of records	Milk yie		
	averaged	Observed	Calculated	Deviation
	89	495-7	492.0	3.7
2	89	443-2	440.8	2.4
3	89	390-4	394.9	-4.5
4	89	351.2	353.7	-2.5
5	89	314.7	316.8	-2:1
6	89	287.7	283.8	3.9
7	89	253.9	254.2	-0.3
8	89	230.2	227.7	-2.5

Ratio of any month's yield to that of preceding month:—'8958.

TABLE IX.

Average monthly rate of milk secretion of Indian buffaloes, with advance in lactation.

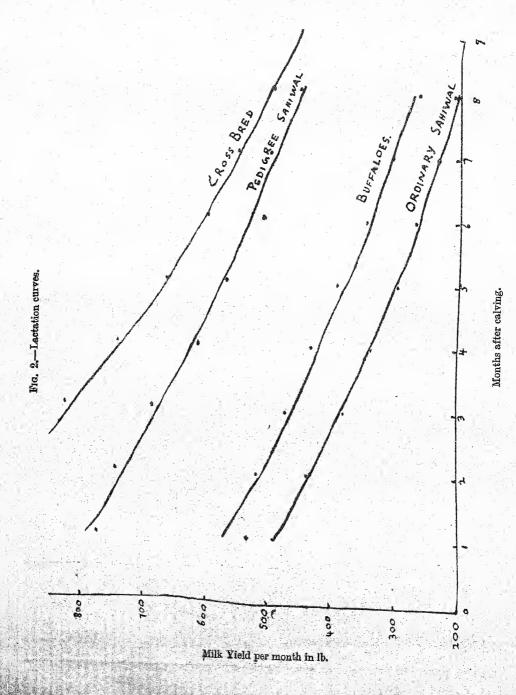
	Month after	No. of records	Milk yie	*'	
calving		averaged	Observed	Calculated	— Deviation
	1	140	531.0	570.9	39.9
	2	137	528-7	520-2	8-5
	3	139	488.4	474.2	14.2
	- 4	139	447-4	432.1	15:3
	5	139	408-0	393.9	14-1
	6	_ 138	363.0	358.9	4:1
	7	134	325.3	327·1	-1.8
Wante	8	112	283-8	298.2	-14·4

Ratio of decline per lb. per month:—09281
Ratio of any month's yield to that of preceding month:—9112

TABLE X.

Yield per month at maximum and the rate of decline per lb. per month for various breeds of animals.

Breed	Yield per month at maximum	Rate of decline per lb. per month	Ratio of any month's yield to that of preceding month
Cross-bred cows	980.9	.09215	-9121
Pedigree Sahiwal	791-1	•0745	9277
Ordinary Sahiwal	492.0	-1101	x A.
Buffaloes.	570.9	•09281	·8958 ·9112



and the constants are tabulated in Table X. The curves for each breed are shown in Fig. 2. From these it will be seen that the smooth curve fits the data very well.

Table X shows that the ordinary Sahiwal cow is the least persistent (i.e., declines most), while the specially bred Sahiwal cows at Ferozepore are most persistent. The buffaloes and the cross-bred are more or less on the same level and are between the other two. (The Ferozepore Pedigree Sahiwals are known to almost equal the cross-bred in milk production. From Table X it will be seen that their maximum is much lower and persistency is much higher than that of the cross-bred. Although these two samples of the two breeds may not be equal exactly, it is still clear that the pedigree animals are able to make up in the course of a lactation a good portion of the difference in their starting yield by virtue of their higher persistency. This shows how important persistency is as a factor in the economical production of milk.)

Two facts might be noticed in the tables that account for a portion of the discrepency between the actual and the theoretical values of the yields. It will be seen that the larger deviations are in the early part of the lactation, which is particularly marked in the case of the buffalo and the cross-bred. This is due to the tendency in many individuals to maintain the rate of yield they attain in the maximum month instead of declining continuously as shown by the theoretical equation. The lactation curve for buffaloes given in Fig. 1 shows this to be the case: here the yield is found to remain steady between the 5th and the 7th week of lactation. Further, the yields as recorded in the tables are from the third week onwards, while the maximum actually occurs a little later in the lactation. The whole of the first month is not, therefore, on the decline portion of the curve, and the theoretical maximum is in excess of the actually realised maximum on account of this as well.

Again, to a certain extent the data are vitiated by the occasional sickness of animals. Such data could be eliminated only when the records contain the remark "sick". Often this is not noted in the records, and if noted, the duration of sickness is seldom mentioned. It is not, therefore, possible to know when such animals have returned to normal health, except by the indication afforded by the restoration of normal milk flow. In certain cases a heavy drop is noticed when an animal falls sick, and this is not made up during the rest of the lactation.

# PRACTICAL APPLICATIONS.

A knowledge of the properties of the lactation curve helps the solution of many problems that are met with in the course of dairy routine and practice. Only one

application is considered here, namely, how it may be tested whether normal conditions of feeding and management exist in a herd and whether the herd has produced the best that it is capable of under such conditions. The other applications will be dealt with separately in a later section.

It has been shown that the yield for a lactation or for a given period may be expressed as a function of the initial maximum rate of secretion and the rate of decline. Two animals may differ with respect to either or both of these characters. For purposes of illustration, Tables XI & XII are given. Table XI shows the monthly yields and total yield for 11 months of animals that have the same maximum but different rates of decline, and Table XII shows animals with the same rate Table XI.

Monthly rate of yield of four typical animals having the same initial rate of yield but different persistencies.

- X	YIELD PRR MONTH IN LBS.									
Time after calving in months.	Type 1.	Туре 2.	Type 3.	Type 4.						
1	1000.0	1000.0	1000.0	1000-0						
2	980.2	941.8	904.8	869.4						
3.	960.8	[886-9	818.7	755.8						
4.	941.8	835-3	740.8	657.0						
5.	923.1	786-6	670-3	571.2						
6.	904.8	740.8	606.2	496.6						
7.	886.9	697.7	548.8	431.7						
8	869.4	657.0	496-6	375.3						
9.	852.1	618-8	449.3	326.3						
0	835.3	582•7	406.6	283.7						
1	818-7	548.8	367.9	246.6						
otal for 11 menths	9973-1	8296:4	7010:3	6013-0						
ate of decline per lb. per month.	•02	•06	•10	•14						
atio of any month's yield to that of preceding month.	.9802	•0418	•9048	-8693						

TABLE XII.

Monthly rate of milk yield of four typical animals having different initial rate of yield but the same persistency.

	Yield per month in lbs.								
Time after calving in months.	Type 1.	Type 2.	Type 3.	Type 4.					
1	400.0	600-0	800.0	1,000.0					
2	361.9	542.8	723.8	904.8					
3	327-5	491·3	655.0	818.7					
4.	296·3	444.5	592.6	740-8					
5	268.1	402-2	536-2	670-3					
6.	242.6	363-9 (	485-2	606.5					
7	219.5	329•3	439-0	548.8					
8	198-6	297-9	397.2	496.6					
9	179.7	269•5	359•4	449.3					
0	162.6	243.9	325•2	406.6					
1	147-2	220.8	294.4	367•9					
l'otal for 11 months .	2,804 0	4,206·1	5,608.0	7,010·3					
te of decline per lb. per month.		1	1	. 1					
tio of any month to that of preceding month.	.9048	19048	•9048	•9048					

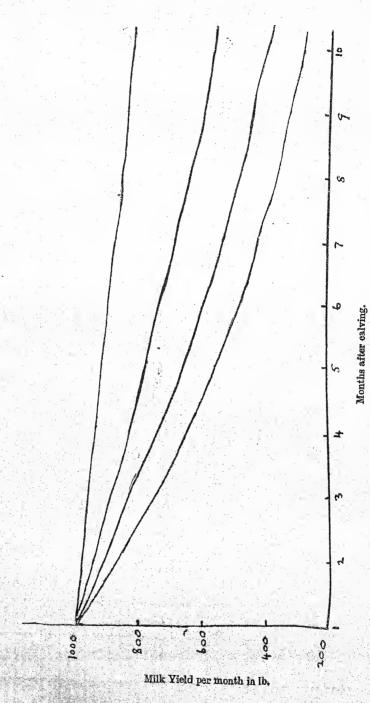
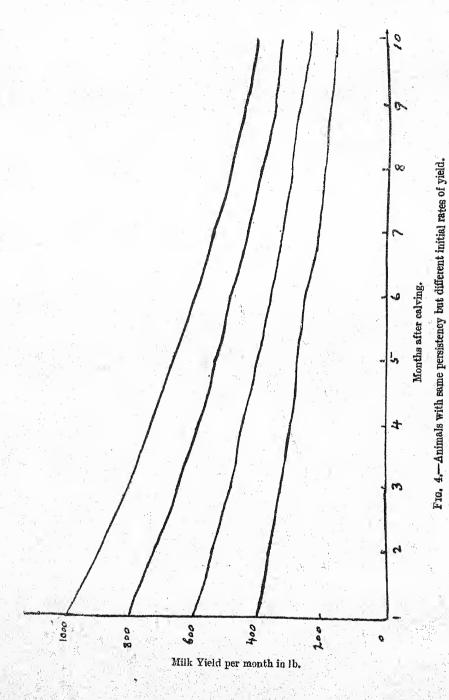
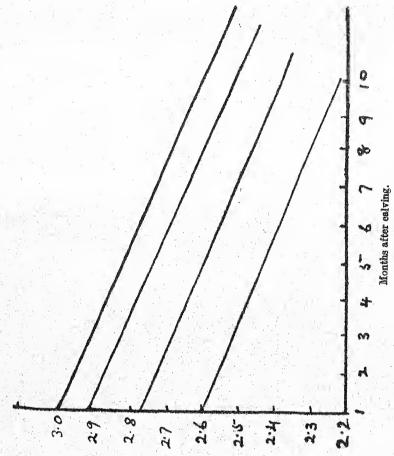


Fig. 3.—Curves for animals with same initial rates of yield but different persistencies.





Logarithms of Milk Yields.

Fig. 3a.-Showing Fig. 3 on Logarithmic scale.

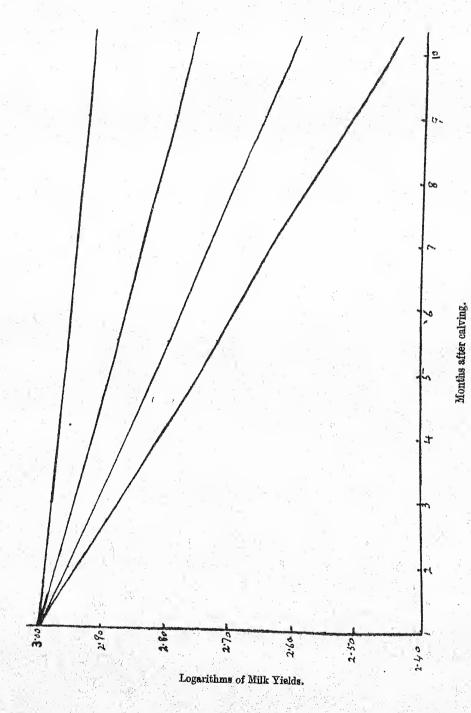


Fig. 4u,--hig. 4 on the Logarithmic scale.

of decline but different maximum yields. Table XII shows that, if the rate of decline is the same, the total yields are proportional to the maximum yields. On the other hand, Table XI shows that with the same maximum the total yield decreases with increase in the rate of decline. When the decline increases from 2 to 13 per cent. or which is the same thing, when the persistency decreases from '98 to '87, that is, by 11 per cent. the yield for 11 months decreases by 40 per cent. This shows, by the way, how important persistency is as a factor in the economical production of milk.

Again, it will be noted that the yield for any month bears a constant ratio to that of the preceding month. In actually observed data the ratios will not be so exactly equal, but they generally tend to equality as has already been observed in Tables I to IX, where the departures from the calculated values were found to be very small indeed, a herd as small as 22 (*Vide* Table IV) giving very satisfactory results. The constancy of this relation makes it possible to test if normal conditions of environment exist in a herd. If  $Y_1$ ,  $Y_2$ ,  $Y_3$ , etc., represent yields for the first second, third, etc., month of lactation beginning with the first full calendar month, the following relation will hold good:—

$$\frac{Y_2}{Y_1} = \frac{Y_3}{Y_2} = \frac{Y_4}{Y_3}$$
, etc.

The value of this ratio will be, as will be seen from Table X, '8958 for the Indian cow, '9121 for the cross-bred and '9112 for the buffaloes. A serious departure from this value indicates the absence of average conditions, and serves as a useful pointer to the dairyman to look more minutely into the feeding and management of his stock.

Figures 3 and 4 show how this can be verified graphically. They represent Tables XI and XII, respectively. It will be seen that the slope of the curve is greater the greater the rate of decline, and they are more or less parallel when the rates of decline are identical. The slope\*, therefore, is a measure of persistency, and by drawing a curve for the herd to be tested by the side of the curve for the herd under average conditions it is possible to see if normal conditions existed.

More accurate results are obtained by drawing the curves on a logarithmic scale. This consists in plotting the logarithms of the yields instead of the yields themselves, or which is the same thing, by plotting them on semi-logarithmic paper instead of on ordinary cross section paper. Figures 3a and 4a are reproductions of Figures 3 and 4 on a logarithmic scale. Here the curves appear as straight lines, and in the case of straight lines the differences in slopes can be more readily appreciated, and exactly measured. If two animals are equally persistent, parallel lines will be obtained. It may be noted in passing that the slopes of these lines divided by 4343 directly give the rate of decline per pound per month. The method of calculation is explained in detail in the Appendix.

<sup>\*</sup>The term slope is used here in a loose, and not in its true mathematical, sense,

The method explained above is particularly useful when reviewing the results of, say, a year's production. Thus if two farms show two different figures of production for herds of the same breed it is conceivable to suppose that the yields have been affected through their constituent, persistency, and if feeding, management and care of the animals had been the same in both the farms the yields would not have been different. Faults in management can thus be easily checked by means of the lactation curve.

In making comparisons like the above, however, due regard should be paid to the economy of feeding. It is possible to show higher persistency by forced feeding and making the animals yield milk beyond the point of greatest efficiency. When this point is passed the food required per lb. of milk is higher than that required on a lighter ration. In this connection the following table may be of interest—it is quoted by Ross, Hall and Rhodes in their study of the feed cost of milk and fat production as related to yields, and it refers to Holtsmark's work based on 846 dairy herds in Norway, owned by practical farmers with the idea of producing milk for profit.

TABLE XIII.

Relation between the amount of feed consumed and the milk produced by 846 dairy herds in Norway according to Holtsmark.

Number of feed units consumed.		× × × × ×	Yield of milk per cow.	Yield of milk per 100 lb. feed units.
	or rest units consumer.		Kilograma.	Kilograms.
1,500 2,000 2,500 3,000 3,500 4,000 4,500			923 1,424 1,813 2,131 2,399 2,632 2,837	61.5 71.2 72.5 71.0 68.5 65.8 63.1

The point of greatest efficiency is reached in the third group. Groups of cows fed more than this amount show decreasing yields per unit of feed.

#### SUMMARY AND CONCLUSION.

498 Milk Records of cross-bred cows, pedigree Sahiwal cows, ordinary Sahiwal cows and buffaloes were analysed for examining at what rate milk yield falls off as lactation advances. It is found that the rate of decline is the lowest in pedigree Sahiwal and highest in the ordinary Sahiwal; the buffalo and the cross-bred are more or less on the same level and come between the two. The maximum rate of secretion is attained at different stages of lactation in different indivi-

duals, but the large majority in all the breeds attain it between the 3rd and the 6th week after calving, that is, roughly in the second month of lactation. The maximum and the rate of decline are as follows:—

				,	Maximum yield per month in lb.	Rate of decline per 100 lb. per month.
**************************************	and district of the same	produce produc	_ 1	-		
٠ . •	•	• -	•		980-9	9.215
•	•,	•.			791-1	7.450
	•		,		492.0	11-010
	•		-		570.9	9-281
						per month in lb.  980.9

How this information may be made use of for computing whole or part of a lactation is illustrated.

Barring minor variations, the yield for any month bears a constant ratio to that of the preceding month. This property enables the dairyman to test if environmental conditions like feeding and management in the herd are normal or not. The ratios for the different breeds are tabulated, and examples are given showing how the lactation curve (i.e., the curve showing the change in the rate of milk secretion as lactation advances) may be drawn for a herd and how the existence of normal conditions may be tested. Examples are also given showing how the best values of the maximum and the rate of decline may be determined from a given set of data.

A table is appended showing the importance of the rate of decline as a factor in the economical production of milk. Here the total lactation yields corresponding to the different values of the rates of decline are recorded.

The writer wishes to acknowledge his deep indebtedness to Captain C. E. Macguckin, M.B.E., Assistant Director of Dairy Farms, Northern Circle, Lahore Cantonment, for the valuable advice and guidance he has received from him in the preparation of this paper.

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#### APPENDIX.

## CALCULATION OF THE RATE OF DECLINE.

In calculating the monthly rate of decline it was mentioned in the text that the yield for the first fortnight was omitted. The monthly yields tabulated are, therefore, successively yields for 5 to 1.5 months after calving, 1.5 to 2.5 months after calving, etc., the mid-points of these periods being 1, 2, 3, etc., months after calving. Thus if time is measured to the middle of the month, we have yields per month at 1, 2, 3, etc., months after calving. As maximum rate of secretion is almost invariably reached between the 3rd and the 6th week of lactation, the yield per month at one month after calving represents the maximum rate of secretion. As the equation made use of represents the lactation curve only from the maximum it would be more convenient to measure time from maximum than from the date of calving.

The attached table shows the details of calculation. Column 1 shows time in months (measured to the middle of the month) from maximum, column 2 shows yields for months given in column 1, the logarithms of the yields are entered in 3; figures in 1 and 3 are multiplied and the product is given in 4. Column 5 is added as a check on the arithmetic, and the figures therein are obtained by multiplying the logarithms in 3 successively by 1, 2, 3, 4, etc. If the arithmetic is correct the totals of columns 3 and 4 when added would give the total of column 5.

In working out the results we are concerned only with the totals of columns 3 and 4. We shall designate these by a and b. In the attached table there are seven observations, from 0 to 6 months. With seven observations, twice the total of column 4 is subtracted from six times the total of column 3, and the difference is divided by 56 and 4343. The quotient gives the rate of decline per lb. per month. Symbolically this may be expressed as  $\frac{6a-2b}{56\times 4343}$ . To find the maximum, three times the difference has to be divided by 8, the quotient added to total of column 3. One-seventh of this sum is the logarithm of the maximum. From logarithmic tables the antilogarithm of this can easily be found out.

Illustration of the method of computation of the rate of decline and maximum.

(Data	:	Ordinary	Sahiwal,	N.	Circle.
-------	---	----------	----------	----	---------

1	2	3	4	5
Time in months from maximum	Milk yield per month in lb.	Logarithm of yields	Product of (1) and (3)	$(t + 1) \times \log y$
T.	y	Log y.	$t \times \log y$ .	*-
0	495.7	2.6952	***	2.6952
1 2	443·2 390·4	2·6461 2·5915	2·6461 5·1830	5·2922 7·7745
3	351.2	2.5455	7·6365	10.1820
4	314.7	2.4979	9.9916	12.4895
5	287.7	2.4590	12.2950	14.7540
6	253.9	2.4046	14.4276	16.8322
Tctal .	. ive	17.8398	52.1798	70.0196

Symbol . . a b

Rate of decline.

6 times total of column 3 (=6a) . . . = $6 \times 17.8398 = 107.0388$ 2 times total of column 4 (=2b) . . . = $2 \times 52.1798 = 104.3596$ 

Difference . ... = 2.6792

This difference divided by 56 × 4343 gives ... 1101

which is the rate of decline per lb per month.

Maximum.

This sum divided by 7 gives 2.6921, which is the logarithm of maximum. The maximum is, therefore, 492 0 lb., which is read from a table of antilogarithms.

With the exception of the last step the details of calculation are the same, whatever the number of observations. In the last step also there is some uniformity; for finding out the difference of the totals of the columns it is always twice the total of column 4 that is to be subtracted, and always the difference has to be divided by 4343. Thus with six observations (0 to 5 months from maximum) the formula for rate of decline would be

 $\frac{5a-2b}{35\times \cdot 4343}$ 

With 8, 9 and 10 observations the formulae are respectively,

 $\frac{7a-2b}{84\times 4343}$ ,  $\frac{8a-2b}{120\times 4343}$  and  $\frac{9a-2b}{165\times 4343}$ 

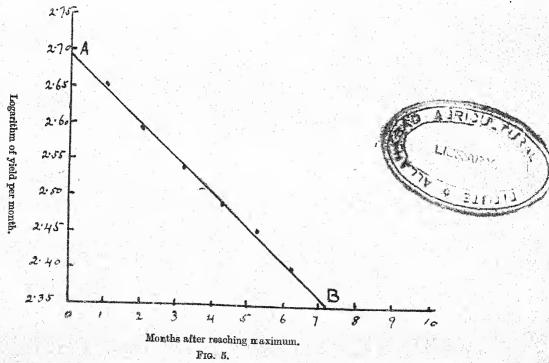
These formulae can be generalised. Thus if n observations are available (0 to n-1 months) the rate of decline is

 $\frac{(n-1) \ a-2b}{4343 \times n(n^2-1)/6}$ 

A general formula may be given for finding the maximum as well. With seven months we took 3/8 of the difference, with 8, 3/9 would have to be taken, and with n observations we have to take 3/(n+1) of the difference. This when added to a and divided by the number of observations gives the logarithm of the maximum.

## Graphical Computation.

Data of yields are collected as before. That is, yields are found between '5 and 1.5, 1.5 and 2.5, etc., months after calving. Their logarithms are then found and plotted as shown in Fig. 5, against time in months as abscissa. A straight line is then drawn to pass as closely as possible to all the points. Very great judgment should be used in drawing this line to see that it is the best fit to the data. Let this line cut the X-axis at B and Y-axis at A as shown in Fig. 5. Measure



Note: (1) OA —Rate of decline per lb. per month.

(2) Antilog of OA is maximum.

OA and OB, and divide the ratio OA/OB by 4343. The quotient gives the rate of decline per month per lb. The working is shown at the foot of the graph. Care should be taken to see that the distances OA and OB in inches are reduced to logarithms and months respectively before finding the ratio.

The logarithm of the maximum is given by OA. Though this method is much simpler, it is less accurate, for a good deal of the accuracy depends upon the care and judgment of the fitter of the line.

## How to draw a lactation curve for a herd.

Yields are generally available for each calendar month of lactation. The yields of the months of calving are omitted, and the average yields for the first, second, third, etc., full calendar month of record are found. These are plotted against time, first observation representing first month, 2nd observation 2nd month and so on. A smooth curve is then drawn passing as closely as possible through all these points.

As anything from 0 to 30 days may have elapsed between the date of calving and the beginning of the first full calendar month of record, the average of the omitted period would tend to be 15 days in a herd, when the herd is not very small. The observed yields are, therefore, yields for the first, second, third, etc., month of lactation when time is measured to the middle of the month; and this agrees with all the foregoing calculations.

## CALOTROPIS PROCERA AND CALOTROPIS GIGANTEA.

BY

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(With Plates VI-XV.)

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#### INTRODUCTION.

Few books have been written on indigenous drugs by medical men in India and fewer still on the drugs used in veterinary medicine. Even in these, most of the matter is either a translation of old literature or hearsay evidence about the uses of the drugs obtained from natives and quacks. In certain cases medical practitioners have described the actions of the drugs which, in their experience, have been found useful in practice and there are just a few which have been investigated on modern scientific lines. Observations based on a few clinical cases are, however, not reliable, as some diseases are cured by nature, independent of the administration of any

medicine, and the particular drug used may be given credit which is unjustified by fact. Moreover if a new drug is administered without experimental knowledge of its actions, it may prove harmful or even fatal in certain cases. In India such effects are not uncommon both amongst human beings and animals in the hands of quacks.

Experimental pharmacology helps to overcome these difficulties. The physiological action of a drug is first studied on healthy animals. Its actions are further studied on various organs on small animals like the dog, cat, etc., with the help of delicate apparatus such as the kymograph.

In the light of the tracings thus recorded and other experimental data similarly obtained a drug can be used with safety and confidence in the treatment of certain diseases. In this way many indigenous drugs having a false reputation may be discarded and useful ones introduced in medicine.

Research work on indigenous drugs is a necessity especially in the veterinary profession for the following reasons:—

Firstly, economy is a great consideration in these days of financial depression and especially in veterinary practice, where the medicines used in the treatment of animals are given in ounces and pounds. Research work on indigenous drugs not only eliminates waste but allows of efficacious country drugs being procurable at the mere cost of collection or at a nominal cost from the bazar.

Secondly, in the villages where allopathic drugs are not available, cheap country medicines can easily be used in the treatment of animals.

Thirdly, in the outbreak of epizootic diseases hundreds of animals get affected at a time and treatment with allopathic drugs is very expensive. There the use of efficacious indigenous drugs helps to slove the economic difficulty. They are likely to be less expensive and easily available.

Fourthly, the results of original research in pharmacology performed on animals may prove unreliable, when applied to human beings but in veterinary work the very animals to be treated are those used in experimental work and the experimental results obtained are thus much more real and reliable.

There is no doubt that the methods of collecting drugs, 'their storage and preservation in the hands of shopkeepers are defective and render many of them useless for medicinal purposes. This can be immensely improved by collecting the drugs at their proper season and time, and preserving them as tinctures, extracts, etc., which delays deterioration of the drugs and preserves the active properties for a considerable time.

#### NOMENCLATURE.

Syn. Sanskrit.—Arka, Alarka, Surya-puttra.—(The leaves of the plant were used in sun worship in Vedic times, hence it is called Surya-puttra or sun-leaf). Sanskrit writers mentioned two varieties founded upon the colour of the flowers, namely, white called Alarka and red called Arka.

Ver.—Madur (1) Hindi, Akanda Bengali, Mandara Bombay, Ak Punjahi, Erruku Tam., Mandaramu Tel., Bijelosha Sind.

### HISTORY.

The ancient name of the plant occurs in Vedic literature as Arka. From one of the sanskrit names of the plant, namely, Mandara, Madar is a corruption (2).

Catotropis Gigantea was first described and figured by Rheeds (2) and an accurate and detailed description was given by Roxburgh at the beginning of the 19th century under the name Asclepias Gigantea.

Robert Brown subsequently showed that it was incorrect to describe this plant as Asclepias, and he founded the genus Calotropis. *Calotropis Procera* was first described from a specimen collected in Egypt by Prosper Alpinus and figured by him on his return to Italy (2).

## BOTANICAL AND SENSORY CHARACTERS.

A middle sized shrub, young parts covered with a pressed white tomentum; bark pale. Leaves subsessile, opposite, broad, obovate or oblong, acute or acuminate, base cordate, cottony beneath. Flowers medium sized simple or compound arranged in axillary or subterminal pedunculate, corolla ½ to 1 inch across, dull-purple or purplish-lilae, or white in colour (1).

Root-bark.—The outer surface is yellowish grey soft and corky, fissured longitudinally and can be easily separated from the middle cortical layer, which is white and friable. The taste is mucilaginous bitter and acrid and the odour peculiar.

Seeds. Ovate 1-inch long with a bright, silk white coma.

# PARTS USED IN THE MEDICINE.

Root-bark, flowers, milky juice and leaves. The part mostly used is the root-bark.

#### COLLECTION.

The roots should be collected in the month of April and May from sandy soil. The older plants should be selected for the collection of the roots, as the older the

Calotropis process is believed by some to be a different variety from the Gigantea, but the medicinal properties are the same,

plant the more active is the bark in its effects. The roots thus collected should be washed to remove particles of sand and dirt and dried in the open air without exposure to sun, until the milky juice contained in them becomes so far dried that it ceases to flow on incisions being made. The bark is carefully removed, dried and reduced to a fine powder.

#### CHEMICAL COMPOSITION.

Dr. Warden [1885], analysing the root-bark at Berlin, obtained white masses of crystals and a yellow resin which were found to agree, as regards their melting point and behaviour, with solvents with Alban and Fluavil respectively obtained from Gutta-percha by Payen, and they were accordingly named Madar-Alban and Madar-Fluavil. He also separated from the drug a yellow bitter resin which is probably the active principle. Dr. E. G. Hill and A. R. Sarkar [1915] analysed the root-bark and have published the result in the Journal of Chemical Society.

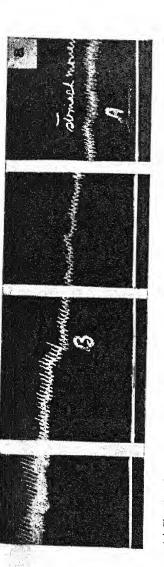
The following is the summary of the results obtained from the quantitative analysis of the powdered bark:—

		Young plant.	Old plant.
Moisture .		12.1	10.2
Spirit extract		15:0	16.2
Soluble in water		7:2	7.5
Resins		<b>7·S</b>	8.7
Total ash		7.0	12.2
Sand		2•8	7.2
Pure ash	*	4.2	5.0

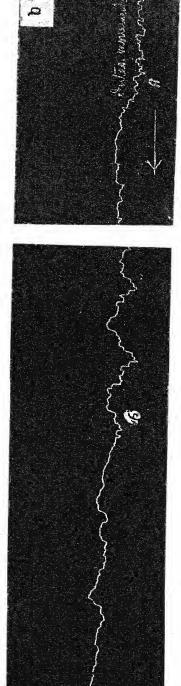
It is clear from the above-mentioned analysis that the root-bark from the older plants has a higher percentage of acrid and bitter resinous matter than that from the younger plants. No alkaloid could be isolated so far from the plant.

# PREPARATIONS.

The roots collected from a sandy soil in the month of May and the bark peeled off and dried according to the instructions mentioned above.

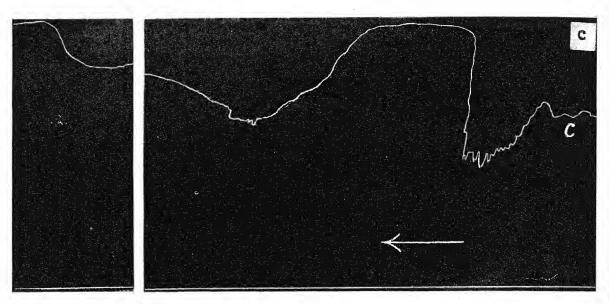


(a) Showing the movements of the stomach of a dog weighing 14 kgms, under chloralose anæsthesia. Note the persistent increase in the tone of the stomach.

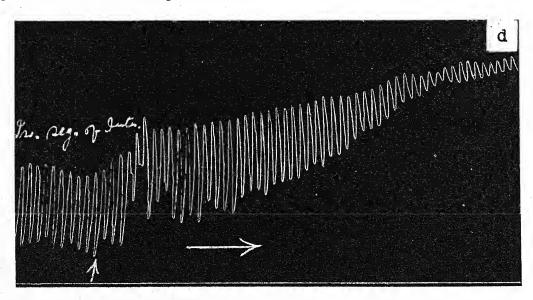


(b) Showing the movements of the intestines of a bitch weighing 11 kgms, under chloralose anæsthesia.

# PLATE VII.



(c) Showing the effect of a bigger dose (0.75 c.c.) of tincture calotropis on the intestines. Note the violent contraction produced under the effect of the drug.



(d) Showing the action of the drug on an isolated segment of rabbits' intestine. Note the marked contraction of the intestinal segment.

The dried bark reduced to powder No. 20 and the following preparations prepared:—

#### Tincture.

Prepared by the process of maceration. (Macerated for a week).

3. Alcoholic saturated solution of calotropis juice.

Mix alcohol 90 per cent, to the equal volume of Calotropis juice and shake thoroughly well. Keep on shaking four or five times a day for a week. Allow it to stand for a few hours and decant the saturated solution of the juice.

#### Physiological action of the drug on animals.

A rana frog weighing 36 gms. was injected with an alcoholic saturated solution of the juice into the anterior lymph sac and the frog showed the following symptoms:—

The respirations became infrequent 20 minutes after the injection. The frog was disinclined to move and there were occasional convulsions in the hind legs. It laid motionless and died an hour after the injection. The heart stopped in systole and was irritable to mechanical and electrical stimuli. Pithing of the spinal cord was followed by convulsions and twitching of the hind limbs.

Tincture calotropis drachm one and alcoholic solution of the juice minims 30 were given to healthy dogs to study their actions.

The symptoms observed on administration are nausea, salivation, vomiting, slowing of the heart and infrequent pulse. The saturated solution of the juice proved very irritant and toxic. It produced an acute gastro-enteritis, restlessness and pain, etc., in addition to the symptoms mentioned above, and the death occurred about an hour after the administration of the drug.

### EXPERIMENTAL INVESTIGATION IN THE ACTION OF THE DRUG.

Externally.—When the juice is applied on the unbroken skin or mucous membrane it acts as a powerful irritant and caustic. Applied to a scarified part or to a cut surface of skin, the juice is a strong irritant and causes a swelling of the surrounding tissues, which is hot and painful.

Internally.—In a dog weighing 14 kgms, under chloralose anaesthesia, the tracings were recorded with the help of an enterograph. A stomach tube was passed and left in the esophagus. 30 c.c. of water administered per stomach tube, as a control, did not produce a marked change, one c.c. of the tincture in 30 c.c. of water given per stomach tube produced a persistent increase in the tone of the stomach. The same dose repeated again gave similar results. (Pl. VI, Fig. a).

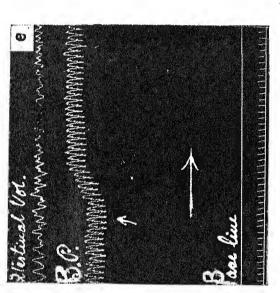
Note the persistent increase in the tone of the stomach in Fig. a at A and B.

The drug increases the tone of the stomach. When given in the form of powder, the calotropis root-bark increases the secretion of the gastric juice by virtue of its being a bitter and thus improves the digestion and increases the appetite. It is therefore a bitter tonic and stomachic. In larger doses it causes nausea and excites the flow of saliva and increases the bronchial secretion. In still larger doses it produces vomiting followed by depression and exhaustion.

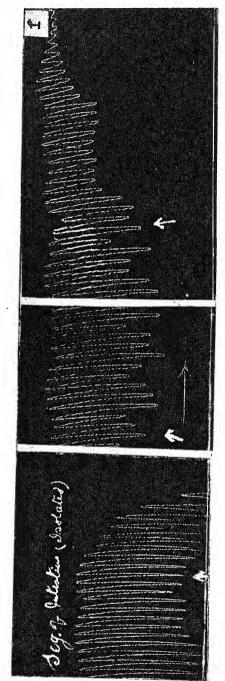
Intestines.—In a bitch weighing 11 kgms, the movements of the small intestines were recorded with the help of an enterograph. The intravenous injection of the tincture in small doses varying from 0.2 to 0.3 c. c. produced an increase in the tone of the intestines and a decrease in the peristalsis. A slightly larger dose 0.4 c. c. of the tincture produced a little relaxation followed by violent contraction of the intestines. There is a relaxation again followed by contraction. (Pl. VI, Fig. b). A still bigger dose of the tincture 0.75 c. c. injected in the femoral vein produced a violent contraction. (Pl. VII, Fig. c.)

Isolated segment of intestine.—An isolated segment of duodenum of a rabbit placed in a bath containing 100 c. c. of Tyrodes' solution being oxygenated. The temperature was kept uniform at 37 C. throughout the experiment. Tineture calotropis 0.1 c. c. added to the bath, where the segment of the intestine was held suspended produced a marked increase in tone. (Pl. VII, Fig. d). The vagal nerve endings of another segment of rabbits' intestine were paralysed in a bath containing 100 c. c. of Tyrodes' solution at 37 C. with atropine. The atropine produced relaxation of the intestinal segment due to vagal paralysis. Atropine solution added to the bath again to ensure complete paralysis of the vagal nerve endings. This failed to produce any further relaxation. The bath washed out and filled again with 100 c. c. of Tyrodes' solution at 37 C. 0.1 c. c. of the tineture added to the bath produced an increase in tone. (Pl. VIII, Fig. f.)

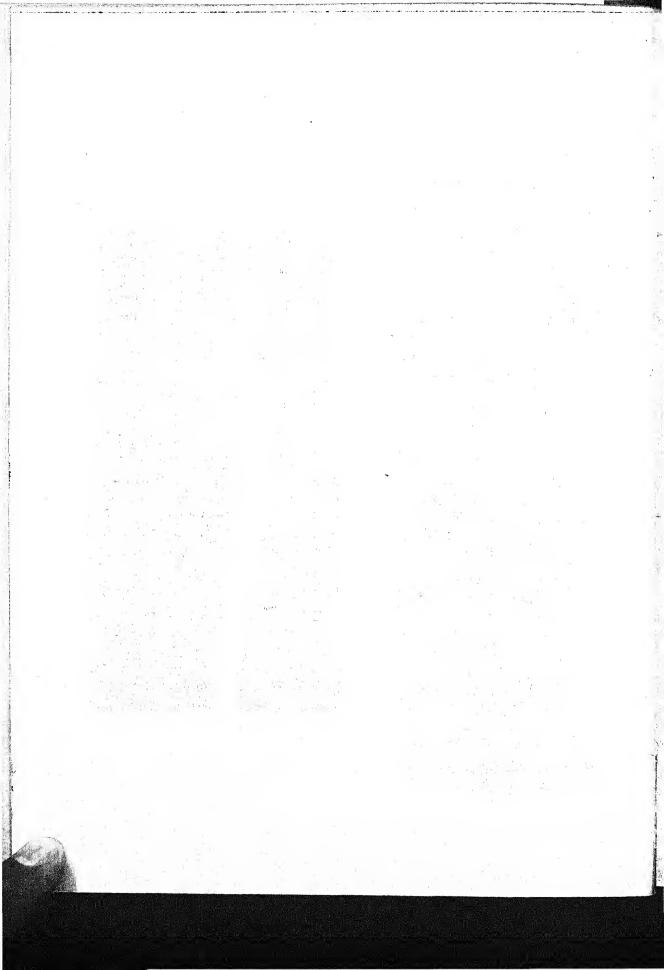
In another experiment a segment of a rabbits' duodenum was kept suspended in a bath containing atropine in Tyrodes' solution for 15 minutes to ensure complete paralysis of the vagal terminations. The solution was allowed to run and to the bath containing 100 c. c. fresh Tyrodes' solution, 0.2 c. c. of the tineture was added. This produced a marked increase in tone which persisted for about 8 minutes. (Pl. IX, Fig. g.)

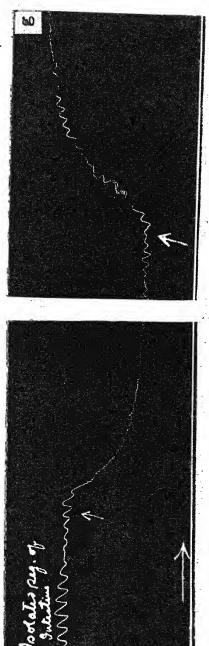


(e) Showing the action of tincture calotropis on the intestinal volume.

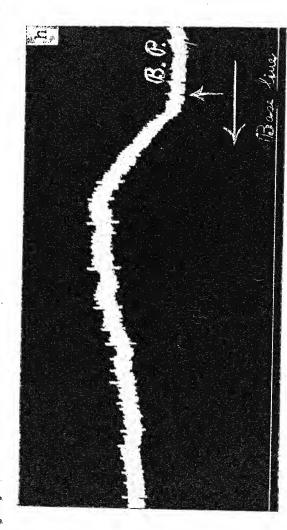


(f) Showing the action of the drug on an isolated segment of intestine after paralysing the vagal nerve endings with atropine.

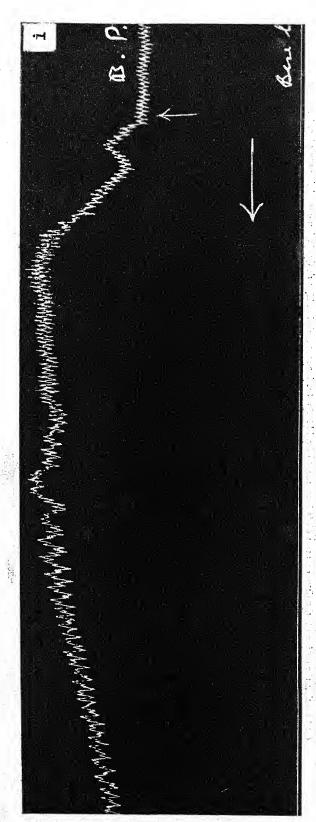




(y) Showing the action of the drug on an isolated segment of rabbits' intestine suspended in Tyrodes' solution containing atropine, for 15 minutes, to insure the complete paralysis of ragal endings. Note the increase in tone on addition of fincture calcing after



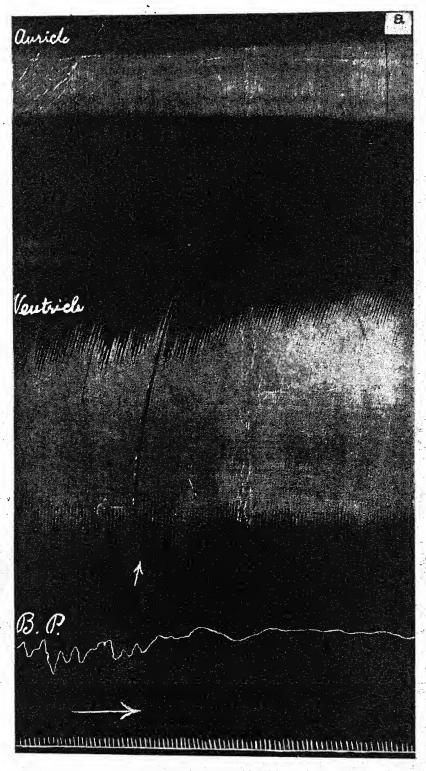
(h) Showing the action of the drug on blood pressure of a dog weighing 17 kgms, under chloretone anæsthesia,



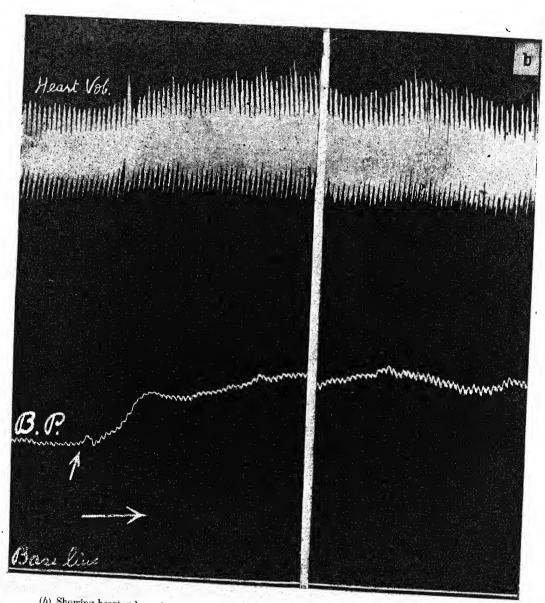
(i) Showing the action of the drug on blood pressure of a dog weighing 12 kgms, under chloretone anasthesia with vagi out,



(j) Showing the action of the drug on an isolated segment of rabbits' intestine. Note the contraction of the intestine produced by tincture calotropis and atropine added into the bath at B and C failed to antagonize its action,



(a) Showing the action of the drug on auricle and ventricle of a bitch weighing 11 kgms. under paraldehyde anæsthesia. The levers move upward during systole, and during diastole downward.



(b) Showing heart volume in an atropinized dog weighing 8 kgms. under paraldehyde anæsthesia.

Another segment of a rabbit's duodenum was held suspended in a bath and the tineture was added and produced a marked increase in the tone of the intestine. At B and C atropine solution was added, which failed to antogonise its action and could not produce relaxation of the intestine. (Pl. X, Fig. j.)

The volume of a loop of an intestine (small) was taken with the help of an oncometer. This remained practically unaffected under the effect of the drug. (Pl. VIII,\*Fig. e).

Conclusion.—Tincture calotropis increases the tone of the intestines both in an isolated segment and when intact. It causes a persistent contraction and decreases peristaltic movements.

After paralysing the vagal nerve endings the tineture produces a marked increase in tone, an action which atropine failed to antagonize. This shows that the increase in tone is not brought about by stimulation of the vagal nerve endings, but most probably by the action of the drug on the musculature of the intestines.

CIRCULATORY APPARATUS.—Blood Pressure.—The blood pressure was recorded in the right carotid artery of a dog weighing 17 kgms, under chloretone anaesthesia.

Injection of tineture calotropis 0.25 to 0.4 c. c. in the dog produced a well-marked and persistent rise of blood pressure varying from 70 m.m. to 140 m. m. of mercury. The blood pressure remained high for about 4 to 10 minutes after which it returned to its normal level, but never went below normal.

In an atropinized dog with both the vagi cut, the rise in blood pressure is more marked. (Pl. IX, Fig. h and Pl. X, Fig. i.)

Heart,—The action of the drug on the heart was studied in a bitch weighing 11 kgms, under paraldehyde anaesthesia. The tracings of auricle and ventricle were recorded with the help of a myocardio-graph and artificial respiration was continued throughout the experiment. Tincture calotropis 0.3 c. c. injected into the femoral vein produced a marked and persistent increase in the force of beats, both of auricles and ventricles. The increased ventricular contractions were more marked. (Pl. XI, Fig. a).

The volume of the heart in an atropinized deg was recorded with the help of cardio-meter. Tincture calotropis injected intravenously showed a marked increase in the volume of the heart. (Pl. XII, Fig. b).

Frogs' heart.—A rana frog was pithed, and perfused with heart in tact, through the inferior vena cava with frog's Ringer solution. The tracings of the ventricles were recorded with a heart lever.

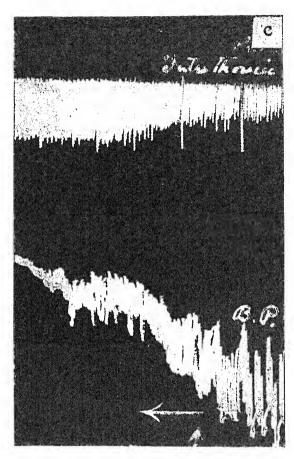
Explanation of tracings is illustrated in Pl. XIV, Fig. d. During systole the lever makes an upstroke, and a down stroke during diastole.

- A. Normal tracing of the frog's heart with Ringer's solution.
- B. The heart was perfused with 0.25 per cent. solution of tincture calotropis in frog Ringer's solution. This produced a slowing of the rhythm and an increase in the amplitude.
- C. The rhythm was slower still and amplitude increased toward systole. The systolic contraction was increased and the dilatation during diastole was greater.
- D. The rhytlim had become very slow and the diastole was much prolonged. The toxic action of the drug has set in.
- E. The toxic action was more pronounced. The action of the heart was very slow, irregular and intermittent, heart block was present and ultimately the heart stopped in systole.

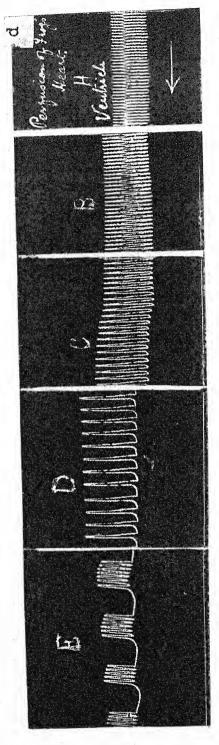
Conclusion.—The action of calotropis on the mammalian heart is to increase the force of the systolic contractions and to cause a persistent increase in the amplitude of contraction and relaxation of auricles and systolic contraction of ventricles. Thus it increases the output of the blood and raises the blood pressure. In the frog's heart it produces an increase in the force of beats and marked slowing of the rhythm. In toxic doses the heart becomes infrequent, irregular and intermittent.

The bronchioles were constricted and there was a marked fall in the blood pressure under the effect of arecoline. Tincture calotropis 0. 4 c. c. injected intravenously produced an increase in the intrathoracic pressure with a rise of blood pressure. This was due to the dilatation of the already constricted bronchioles (under the effect of arecoline) and consequent increase of the volume of the lungs. (Pl. XIII, Fig. c.)

Uterus.—An isolated strip of rabbits' uterus was suspended in a bath containing 100 c. c. of Tyrodes' solution at 37° C. Tinct ure calotropis 0. 1 c. c. added into the bath at the arrow mark (Pl. XV, Fig. e) produced a strong contraction of the uterine strip with incomplete relaxation following. The stimulating action occurs in all animals, whether pregnant or not, and the action is attributed to direct effect upon musculature.



(c) Showing the action of tineture calotropis on the intrathoracic pressure of a dog weighing 11 kgms, under chloretone anæsthesia. The bronchiols were already constricted under the effect of arecoline.



(d) Showing a frog's heart intact perfused with 0.25% solution of tincture calotropis in Frogs' Ringer solution. During systole the lever makes an upstroke and downstroke during diastole.

40 m.

## Toxicology.

The milky juice of the plant is very acrid and is used for criminal purposes as an abortifacient and as an infanticide in human beings (5). When applied on the raw surface or over a freshly cut skin, it is absorbed and produces an acute inflammatory swelling of the surrounding tissues and may produce general poisoning. The milky juice is sometimes used in mallacious poisoning of cattle.

The following experiments were undertaken to study the toxic action of the drug:-

#### T.

Subject.—A healthy dog weighing 8 kgms.

2-30 P.M. pulse 92 per minute.

2-35 , The following was given by stomach tube:—

Alcoholic saturated solution of Calctropis juice 2 oz . Aqua .

- 2-40 P.M. The dog walks about, cries and is restless. Pulse 105 per minute.
- 2-50 , Nausea, attempts at emesis and vomited twice. Pulse 105 per minute.
- ,, Vomiting continued. The animal arches the neck, lowers the head and makes several attempts at emesis but only vomits a white foamy liquid. Pulse 90 per minute.
  - 3-5 P.M. Passed semiliquid faeces. Pulse 83 per minute.
- 3-15 , The animal looks depressed. Dribbling of saliva from the mouth. Occasional attempts to vomit. Pulse 75 per minute.
- 3-25 P.M. The animal prefers to lie down and shows a great desire for water, which however is quickly rejected on drinking. Pulse 68 per minute.
- 3-35 P.M. Dribbling of saliva continued and the animal remained lying for most of the time. Pulse 65 per minute. The animal looks very much exhausted.
  - 3-45 P.M. The dog keeps lying down and pulse 90 per minute.
- 4-15 ,, The dog lost control over the hind limbs. Pulse very much accelerated, weak and almost imperceptible. The animal died after a few convulsive movements at 4-18 P.M.

Post-mortem report—Acute gastro-enteritis with excoriations of the mucous membranes.

Congestion and fatty degeneration of the liver.

#### II.

To another dog weighing 8 kgms, alcohoric saturated solution of the juice 15 m. in 4 dr. of distilled water was administered subcutaneously. The animal showed the following symptoms:—

After half an hour's time the dog became restless, jumped and cried. Respirations accelerated and laboured and profuse salivation from the mouth. Pulse infrequent, later irregular and intermittent. The animal died an hour and three minutes after the injection.

Post-mortem revealed an enteritis of the small intestines, fatty degeneration of the liver and endo-carditis close to the apex in the left ventricle.

#### III.

The third dog weighing 9 kgms, was injected with 15 min, of alcoholic saturated solution of the juice in 4 dr. of sterilized distilled water intra-peritoneally. The animal uttered a few cries, walked round, dribbling of saliva from the mouth and fell down. The pulse was weak and imperceptible and soon the heart stopped beating. The animal took a few gasps and died 7 minutes after the injection.

#### 1V.

Donkey male c. b. mouse-coloured 10 years.

The alcoholic saturated solution of the juice 1 dr. injected subcutaneously. The animal showed the following symptoms:—

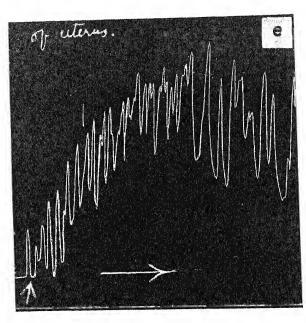
After 15 minutes there was perspiration all over the body particularly over the scrotum, neck and ears.

Respiration accelerated. Later the animal became restless, bathed in perspiration, lost control over the hind limbs and fell down. The animal tried to get up but could not do so and strikes its head against the ground. Respiration infrequent, pulse weak and frequent, neighing frequently. The animal kept lying down, respiration deep and occasional, pulse very weak and almost imperceptible. The donkey died an hour and eight minutes after the injection.

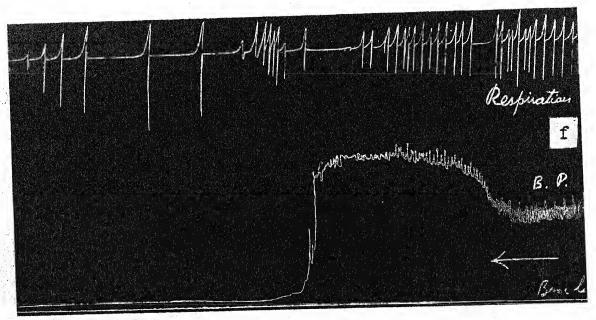
Post-mortem. Liver highly congested, small intestines inflamed and kidneys congested.

A dun horse 7 years old was admitted as an in-patient on the 4th June 1931. The animal had a contusion of the off shoulder. The owner treated him by applying calotropis juice over the shoulder after scarification, which produced symptoms of poisoning. There developed a huge painful swelling on the shoulder.

PLATE XV.



(e) Showing the action of the drug on an isolated strip of a rabbit's uterus. Note the marked contraction under the effect of the drug.



(f) Showing the toxic action of the drug on a dog weighing 12 kgms. under chloretone anæsthesia. Note the sudden fall in the blood pressure due to stoppage of the heart under the toxic effect of the drug.

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breast, forcarm, neck and extending upto the head. The animal was restless and could not bear weight on the off forc leg. The swollen parts pit on pressure and the prescapular lymph gland was enlarged and swollen.

Respiration and pulse accelerated. Temperature ranged between 102° F. and 103.4° F.

The restlessness increased and the nervous symptoms developed, the animal striking his head against the ground and remained lying most of the time. There was trembling of the muscles and the animal was in a great distress. Died on the Sth Jane 1931.

To study the toxic action of the drug further, the respiration and blood pressure were recorded in a dog. The alcoholic saturated solution of the juice 0.5 c. c. injected into the femoral vein first raised the blood pressure and then brought it down to zero. The heart stopped beating and the respirations became irregular and intermittent but they continued even after the heart stopped. (Pl. XV, Fig. f.)

The juice is very irritant and corrosive poison. It produces an acute gastroenteritis when given by mouth. When applied on a scarified skin over a large area or over a raw surface, it produces an extensive, painful swelling. It acts particularly on the small intestines, liver and heart after absorption. There is a very little danger of its poisoning when applied on an indolent ulcer or over chronic yoke and saddle galls. This is due to the fact that in indolent and long standing ulcers a lot of fibrous tissue is formed, which checks its absorption and thus the action remains localised to the part where it is applied.

Antidotes. In cases of poisoning by calotropis, demulcent and mucilaginous drinks such as milk, rice gruel, etc., should be given and morphine and atroping administered to allay the pain. If the poisoning is due to absorption from the skin, the local treatment consists in applying cold water to the swollen pain followed by Glycerine Belladonna or some such soothing preparations. If the juice is sticking to the part, it should be well washed and the juice thoroughly removed before any other application is made to the part. If there is a danger of heart failure diffusible stimulants and heart tonics such as ether or camphor are indicated.

Discussion. Tinctures of the drug were prepared both by percolation and maceration, according to the directions of the British Pharmacopea, but those prepared by maceration were found pharmacologically more active and a tincture prepared in this way was used throughout the experiments in order to maintain uniformity.

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The alcoholic saturated solution of the juice, being highly poisonous, is never used in ordinary experiments. This is used only when toxic action of the drug is being studied; as the juice is the common cause of poisoning. The decoction has been found to have slight pharmacological activity.

#### Doses.

Tincture calotropis increases the tone of the stomach and intestines. Both pharmacologically and in toxic doses the drug appears to have a selective action on the liver and small intestines, whatever channel of administration is used. The action on the intestines is not by way of the vagal nerve as it persits even after paralysis of the vagal nerve endings with atropine, while the contraction produced by tincture calotropis cannot be antagonized by atropine. The drug appears to act on the musculature of the intestines.

It causes a slowing of the pulse and at the same time increases the force of the heart and raises blood pressure.

Doses	s.—(per mouth) Root-bark powder.	Dog		2 to 8 gr.
		Horse and cattle		1 to 3 dr.
	Tincture of calotropis root-bark.	Dog	•	5 to 15 m.
		Horse and cattle	•	2 to 3 dr.
	Subcutaneous injection in a drachm of sterlised distilled	Dog	•	2 to 8 m.
	water.			5 4 42
	Intravenous injection diluted in 2 c. c. of distilled water.	Deg		2-5 m.

(The biggest doses of the tincture given subcutaneously produced localised swelling.)

My thanks are due to W. Taylor, Esq., M.R.C.V.S., D.V.H., I.V.S., Principal, Punjab Veterinary College, Lahore, for his kind advice in connection with this work.

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# PRELIMINARY NOTE ON THE USE OF GOAT TISSUE VACCINE ALONE FOR THE CONTROL OF OUTBREAKS OF RINDERPEST.

BY

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AND

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(Received for publication on the 5th February 1934.)

# INTRODUCTION BY P. J. KERR, I.V.S.

A brief introduction is necessary to this note on the use of goat tissue vaccine for the control of outbreaks of rinderpest. On the advice of Dr. Edwards, Late Director, Imperial Institute of Veterinary Research, Muktesar, goat virus has been used in Bengal for immunisation of cattle by the Serum Simultaneous method since 1929. The behaviour of the controls used in these operations led us to believe that goat virus alone might possibly be used with safety. Experiments on a small scale were carried out to demonstrate this point, with most encouraging results, but had to be closed down for want of funds and staff.

It was not known to me that Menon had already experimented with goat virus alone on a small scale in 1927-28. This work was referred to by Major Stirling in his report, 1932. In the meantime this work was taken up independently in the Central Provinces by Major Stirling and in Mysore by Major Simpson. The results of these officers amply demonstrated the safety of this method for plains cattle which had not been subject to the risk of natural infection. It appeared to me that the next step was to test the application of this method in the face of the disease and also its safety for buffaloes, if full benefit of the method were to be obtained.

In my opinion there are several objections to the wholesale immunisation of cattle against rinderpest.

(1) An effective campaign on these lines is not possible with the veterinary staff employed in India.

- (2) The immunisation of all the scrub cattle in India is not desirable, were it possible.
- (3) It is only in face of an outbreak that all susceptible animals must be immunised, as, good or bad, they can act as a source of infection and spread the disease.
- (4) To apply virus alone inoculation in the absence of the disease is liable to bring the method into disrepute, as ignorant owners are apt to opine that we have introduced the disease for our own purposes should any serious reactions occur.

Before putting the goat virus alone method in face of the disease into practice on an extended scale I went to Muktesar to discuss the various aspects of the matter with Mr. Ware, the Director of the Institute, and Mr. Haddow the Scrologist. I learnt that the latter would shortly be prepared to issue goat tissue virus instead of goat blood virus. For the purposes of this note I have adopted the name of goat tissue vaccine for the former product, tissue vaccine for short.

On the appointment of Mr. Menon to Bengal as Veterinary Investigation Officer, I decided to utilise his long experience and special knowledge of rinderpest work at the Institute, and to concentrate on this problem of the use of virus alone to control outbreaks of rinderpest.

### GOAT VIRUS ALONE IN FACE OF THE DISEASE.

In the first instance goat blood virus was used. The seed virus being obtained from Muktesar from which the virus for injection was freshly prepared in the field. 1774 inoculations were performed in this way with very encouraging results:—

	-	Reactions.	Deaths.
Cattle 1	765	: 58=3.3%	10=0.57%
Buffaloes	9	: 4-44%	Nil

In each case the outbreaks were controlled but in the course of this work it was evident that this method would not be suitable for general application in place of serum, if goat blood virus were used.

- 1. The technique of this method was beyond the ability of the average Veterinary Assistant Surgeon to carry out singlehanded.
- 2. It necessitated the presence of a more experienced officer particularly with regard to the interpretation of the reaction in the virus producing goats.
- 3. There was unavoidable delay of 3-4 days after arriving at the seat of outbreak while the virus was under preparation.

4. There were difficulties in regard to sterilisation of the appliances and instruments under field conditions.

It was also impracticable to maintain at headquarters a large stock of freshly prepared blood virus to issue in place of serum.

# GOAT TISSUE VACCINE IN FACE OF THE DISEASE.

We decided to continue the work using goat tissue vaccine. The advantages of tissue vaccine are:—

- 1. It is easy to prepare at headquarters, preserve in scaled ampoules, and keep in cold storage till required.
  - 2. These ampoules are handy for packing and despatch by post.
  - 3. The vaccine can be ready for injection one hour after receipt.
- 4. One goat produces 2,000 to 2,500 doses as compared with some 500 doses of blood virus (Small Bengal goats).
- 5. It retains its potency in cold storage at 45° F for 30 days after preparation and for 7 days at room temperature after removal from cold storage, under cold weather conditions. (Maximum temperature 85°F.)

Several preliminary tests had to be carried out to ascertain the above facts as to viability of the vaccine. The tests of the viability of the vaccine in cold storage were carried out at the Bengal Veterinary College during October, 1933. Then owing to lack of facilities to carry on this work there, the subsequent tests had to be postponed until a temporary vaccine depot had been established elsewhere. These tests were eventually carried out during the X'mas holidays at Barrackpore.

The spleens of the viability experimental animals were ampouled and issued to selected officers in the district for the control of outbreaks of rinderpest, in place of serum. From the establishment of the Barrackpore depot at the beginning of December the regular preparation of vaccine was commenced.

In all some 10,764 animals while subject to natural infection have been inoculated with most satisfactory results. The outbreaks were stopped. Buffaloes were inoculated together with the cattle.

Cattle		10,119	reactions .	167=1.6 %	deaths .	51=0.5 %
Buffaloes		645		52=-8 %		Nil.
		10.764		***************************************	1.0-	-
	**************************************	200704		219=2.0 %		51=0.47 %

The seats of outbreak were inspected by responsible officers 5 to 6 days, and 10 to 11 days after the inoculation to ascertain results. Animals were judged to be reactors if they showed clinical symptoms of fever. We personally inspected as many inoculated animals as possible and our observations tally for the most part with those of other officers. Taking into considerations the large number of animals inoculated, and the readiness of owners to report unfavourably upon any new method of treatment we feel that the reporting is reliable. Any error being against rather than for the new method.

Over 3,000 animals in controlled herds were done under our personal supervision and subsequent inspection. In these no hearsay evidence was taken and the results were more favourable than generally reported by other officers.

The selected localities for trial of this method were as widely separated in the province as the incidence of disease permitted. We were also handicapped to some extent, till X mas, by our uncertainty as to the viability of virus after issue from cold storage. It had been demonstrated potent up to 4 days, but this was not long enough for its use in the more remote districts to reach which a parcel takes three days by post.

The need for the careful application of this method in each district of the province has been appreciated as the incidence of reaction is far from uniform, and susceptibility may be higher in submontane tracts from the mixture of hill blood.

It was not possible to take the temperatures of all animals inoculated, and some few were done while in the incubation period of the disease. These have, however, been included in our main calculation of results, but subsequently abstracted.

It was not possible either to take temperatures of all reactors reported. It was expected that buffaloes would show a higher percentage of reactions and this was found to be the case. In one instance all the male buffaloes inoculated showed a strong reaction, while the females reacted normally. This must be put down to the fact that all the males were put to work immediately after injection and kept at work until they went sick. No fatality occurred. In order to modify the reaction in buffaloes, if possible, the dose was reduced to ½ c.c. after this experience.

To test the immunity conferred by giving ½ c.c. doses, two buffaloes were tested four weeks after immunisation by giving them 1 c.c. of fresh bull virus obtained from a natural case of rinderpest. No reaction whatever was shown. Similarly the immunity conferred by giving cattle 1 c.c. of tissue vaccine was tested by giving 1 c.c. of fresh bull virus taken from the same animal. No reaction

occurred. Twenty animals were selected for this test, 16 cattle and 4 huffaloes. The animal from which the virus was taken died of rinderpest.

All the animals immunised in this herd were allowed to mix freely with diseased ones and no case of post-inoculation infection occurred.

The theoretical danger of animals contracting the disease during the negative phase appears to us to be negligible. In our experience cases of sickness after injection have been genuine reactions to the inoculation or animals inoculated during the incubation period of the disease. In our opinion the small quantity of attenuated vaccine injected has not had any definite influence on the course of the disease in these cases.

# PREPARATION OF THE VACCINE FOR USE IN THE FIELD.

The ampoules sent out contain approximately 1 gram of spleen tissue in each. This is prepared by removing the tissue and placing it in a glass mortar and emulsifying with 0.75 saline solution. The saline is added gradually and decanted at intervals until 100 c.c. have been used and the tissue thoroughly emulsified. This process takes fully half an hour and at the end there should be only small particles of fibrous tissue remaining in the mortar. We use an enamelled mug with a lid to contain the emulsion to protect it from sunlight and dust. Small quantities are decanted into a Petri dish for inoculation as required. The emulsion must be used on the day it is prepared and be protected from excessive heat and sunlight.

In conclusion we wish to refer briefly to the work on goat virus done by Dr. J. T. Edwards while at Muktesar. In the first instance the passage of bull virus through goats was carried out with the object of removing piroplasms. After a series of about 30 passages he discovered that it had acquired a very definite degree of attenuation also. In view of this he hoped to work out a satisfactory vaccine for use against rinderpest in place of serum alone and Serum Simultaneous methods of inoculation. Mr. Haddow continued the investigation work and evolved the spleen tissue vaccine under report. From the results now published it seems that this goat spleen vaccine fulfills the hopes of both investigators to a very great extent. It is realised that the tissue vaccine has only been applied to plains animals in Bengal and that it must be tentatively applied to other breeds in other provinces, but from the known results of the work with goat blood virus in other provinces, notably in the Central Provinces and Mysore, there is every reason to expect results similar to those in Bengal. Should this be the case, the application of goat tissue vaccine alone will revolutionise the methods of control of rinderpest in India.

TABLE I.

Summary of goat wrus (blood) alone inoculation in the scene of outbreak of rinderpest in Bengal, 1933.

A Constituted A	Number incoulated.	Reactors.	Death.	Romarks.
Cattle	1,765	58=3·3 %	10=0.57 %	Details of reaction after inoculation. 2nd day 1 cow. * 3rd , 18 cows. * 7th day and upwards 39 cows 2.2%
Buffaloes	9	444 %	Nil	Details of deaths.  † 5th day after inoculation 2 cows.  7th ,, ,, , 8 cows.  0.45 %
Total	1,774	62=3.4 %	10=0.57 %	, '

N.B.-\* Out of 58 reactors 19 appear to have been vaccinated during the normal period of incuba-

+ Out of 10 deaths 2 died on 5th day after vaccination and were probably already infected at the time of inoculation.

TABLE II.

Summary of rinderpest tissue vaccine (goat) inoculation in the scene of outbreak of rinderpest in Bengal, 1933-34.

<u>-</u>	Number inoculated.	Reactors.	Deaths.	Remarks.
Cattle	10,119	167=1.6 %	51=0.5 %	Details of reaction after vaccination.  1st day 1 animal 2nd , 5 animals 3rd , 16 , Remainder 145 are the actual reactors to vaccine=14 %
Buffaloes.	645	52=8.0 %	Nil.	Details of deaths.  5th day . 4 died .  6th ,, . 2 ,,
Total	10,764	219=20 %	51=0.47 %	

N.B.—\*Out of 167 reactors 22 appear from the records to have been vaccinated within the normal incubation period.

†Ont of 51 deaths 14 appear to have been within the normal incubation period when vaccinated.

# SELECTED ARTICLES

# RABIES. A REVIEW OF RECENT ARTICLES, XIX.\*

BY

#### A. G. MCKENDRICK.

I Reprinted from Tropical Diseases Bulletin, Vol. 30, No. 9, September 1933.]

#### 1. VIRUS.

In a fully illustrated paper Levaditi, Schoen and Mezger¹ collect the researches already summarized (this Bulletin, Vol. 30, p. 134), from communications to the Bull. Acad. Med. and the C. R. Soc. Biol. They hold the opinion that the "parasite of rabies" should be called Glugea Lyssae, and not as has been proposed by Manouelian and Viala, Encephalitozoon rabiei. The morphological appearances in no way resemble those of Encephalitozoon cuniculi. The spores of the latter have a uniform appearance, and their volume in rabbits and mice is invariable. Their structure is perfectly definite and so also are the cysts or pansporoblasts which enclose them. The oxyphil corpuscles described by Levaditi and his co-workers in rabies infections of mice, though they resemble in certain stages those described by Manouelian and Viala in the rabbit and the dog, are in reality pansporoblasts consisting of an infinity of sporules of dimensions which are almost ultramicroscopic. The sporules do not posses definite characters at all comparable with the spores of Encephalitozoon cuniculi.

A further study of the cystic stage of 'Glugea Lyssae' in the white mouse has been made by Levaditi and Mezger<sup>2</sup>, using dark ground illumination with a magnification of about 1,800 diameters. The cysts measure from 5 to 20 micro-millimetres. They are covered by a refractile membrane, and are round or ovoid. These sacs contain the young cysts whose number is proportional to the size of the envelope. The young cysts are also covered by a refractile membrane, they enclose a dark substance, and are separated from each other by granular material.

<sup>\*</sup> For the eighteenth of this series see Trop. Dis. Bull. Vol. 30, pages 133-143.

<sup>&</sup>lt;sup>1</sup>Levaditi (C.), Schoen (R.) and Mezger (J. G.). Etude morphologique du virus rabique. Arch. Internat. Med. Experim. 1932. Dec. Vol. 7, No. 4, pages 655-692. With 21 figs. and 1 coloured plate. (Refs. in foot-notes).

<sup>&</sup>lt;sup>2</sup>Levaditi (C.) and Mezger (J. G.). Structure polykystique du parasite de la rage. C. R. Soc. Bioi. 1933, Feb. 10, Vol. 112, No. 5, pp. 440-442. With 1 fig.

That the 'noyau optique basal' of Nicolau and Kopeiowska and what Thomas and Jackson call the 'nucleus oculomotorius' is a seat of election for Negri bodies has been already referred to (Vol. 28, p. 744 and Vol. 29, p. 600). [There is no doubt in the reviewer's mind that the seat of election described by the latter authors in October 1930 is the same as that described by the French authors in May 1932]. The French authors, it will be remembered, found Negri bodies in this region in fixed virus rabies of rabbits. They confirm this observation in the case of two strains of fixed virus, one a virus of Chien fou isolated by Mathis and Constantinesco (Vol. 29, p. 597), and the other a highly virulent strain of fixed virus isolated by Jonnesco in Rumania (Vol. 30, p. 133). In the case of the Mathis strain the inclusions are much more numerous and of larger dimensions in the seat of election than in the horn of Ammon. In the case of the Rumanian strain they are so small and rare as to be practically non-existent in the horn of Ammon, whereas in the elective zone they are numerous, of large dimensions, and show the characteristic internal structure.

A full account of the occurrence of rabies in the Union of South Africa is given by Neitz and Marais<sup>4</sup>. The summary by Dutoit quoted in this Bulletin, Vol. 28, pp. 742-743, brought the history up to 1928. It now appears that since that date the existence of rabies has been proved in thirty instances, and presumed on clinical evidence in six other instances. Of these, eight have occurred in the human subject. As before genets and meerkats appear to be the most usual source of infection. Photographs of these animals are given, and also maps showing the distribution of the disease. The probable origin of the infection is discussed, and methods of control, adopted and proposed, are considered.

It will be remembered that Vaucel, Boisseau and Saleun proved by cross immunity experiments that a strain of street virus from Brazzaville (French Equatorial Africa) was identical with the rabies virus of Europe. Vaucel and Saleun<sup>5</sup> have now studied three other strains of virus from the same vicinity. In each case they have experienced no difficulty in passaging the strain through many generations, and in two, crossed immunity experiments have shown that the viruses were identical with rabies. "In spite of the diversity and indefiniteness of the

<sup>&</sup>lt;sup>3</sup>Nicolau (S.) and Kopelowska (L.). Rage du lapin, à virus fixe, et corps de Negri; dénombrement comparatif des inclusions dans la corne d'Ammon et dans la zone elective (noyau optique basal). C. R. Soc. Biol. 1933, Feb. 10, Vol. 112, No. 5, pp. 445-448. With 2 figs.

<sup>\*</sup>Neitz (W.O.) and Marais (I.P.) Rabies as it occurs in the Union of South Africe. Union of South Africa 18th Report, Director Vet. Services and Animal Industry, Onderstepoort, Pretoria, 1932. Aug. pp. 71-93. With 5 figs. and 6 maps, 32 refs.

<sup>&</sup>lt;sup>5</sup>Vaucel (M.) and Saleun (G.). Rage canine en Afrique Equatoriale Francaise. Bull. Soc. Path. Exos. 1932, Dec. 14, Voi. 25, No. 10, pp. 1018-1021.

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symptoms observed in the animal, the three strains which have been studied have behaved, from the histological point of view, like those of true rabies."

An exceptionally virulent strain of street virus is described by Palawandow and Serebrennajac. It was obtained at the Odessa Institute from the saliva of a twelve-year-old girl, bitten by a dog on the leg. She had received no treatment and developed rabies after an incubation period of two months. The first passage of saliva intramuscularly into a guineapig gave an incubation period of 12 days. In cleven subdural subpassages in guineapigs the incubation varied from 1 to 4 days and averaged about 2½ days. The numinal lethal dose was 0.2 cc. of a 1 in 75,000 dilution. The virus is now in its 40th passage. After the eleventh passage a feature of extraordinary interest was noticed that the incubation periods gradually lengthened. From the 10th to the 29th passages the incubation for guineapigs was 3-32 days, and for rabbits 3-4 days. After the 29th the incubation period for guineapigs was 4-6 days, and for rabbits 5-7 days; in fact the virus had become fixed virus. (It is impossible to reconcile these facts with the view which is usually held that fixed virus is a virus which has become accommodated to the easier mode of transmission from brain to brain, whereas street virus is accustomed to a much more complicated transmission from saliva to nerve-endings and central nervous system and vice versa at each passage. The authors assume that in the case of the Sokolan strain (described above) the virus comes to equilibrium as a fixed virus, after the elimination of some accelerating influence.)

The strain of fixed virus employed at Budapest has been examined by Aujeszky and Kerbler.<sup>7</sup> This virus was taken from a dog by Högyes in 1886 and is now in (approximately) its 17,000th passage. The dose which will kill with certainty when injected subdurally is 0.15 ee. of a dilution of 1 in 6,000, that is to say 0.025 milligrammes of brain substance. The incubation period has shortened—paralysis on the 5th day—and the period of illness has also diminished.

In a previous communication (this Bulletin, Vol. 29, p. 189), Shope draw attention to the close resemblance between the virus of the "mad itch" of cattle in America, and the pseudorabies of Anjeszky. He has now<sup>8</sup> carried out cross immunity experiments between a "mad itch" virus obtained from a cow in Iowa, and a pseudorabies virus furnished by Aujeszky from Budapest. The various

<sup>&</sup>lt;sup>6</sup>Palawandow (Haidar) and Serchronneja (A. I.). Ueber einig Eigentumlichkeiten der im Odessaer Bakteriologischen Institute isolierten Strassenvira. Zischr. f. Immunitalsf, u. Experim. Therap. 1933. Vol. 78, No. 3-4, pp. 230-238, 12 refs.

Aujeszky (A.) and Kerbler (F.). Ueber Veranderungen der biologischen Eigenschaften des Virus fixe. Giorn. di Batteriol. e Immunol. 1933, Feb. Vol. 10, No. 2, pp. 257-266, 10 refs. Erglish summary.

<sup>\*</sup>Shope (Richard E.). Identity of the Virus causing 'Mad Itch' and Pseudorabies. Proc. Soc. Reperim. Biol. and Med. 1932, Dec. Vol. 30, No. 3, pp. 308-309.

84 THE INDIAN JOURNAL OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY [ IV, 1. mixtures of serum and virus were stored in the refrigerator over night. The results were as follows:—

Virus.	Serum.	* = 5	No. of Guineapigs.	No. died.
Pseudorabies	Normal		5	<b>5</b>
	Pseudorabies convalescent		6	0
· · · · · · · · · · · · · · · · · · ·	Mad itch convalescent		4	0
Mad itch	Normal		5	5
•	Pseudorabies convalescent		6	0
99	Mad itch convalescent .		4	0

1 cc. of a 10 per cent, suspension of virus was mixed with 0.5 cc. serum from a pig; 1.5 cc. of the mixture was inoculated subcutaneously. "In recognition of the identity . . . the mad itch virus will in future be designated as Pseudorabies Virus (Iowa strain)."

In a second communication 9 Shope continues his study of this strain. The virus was passaged separately through the brains of 9 guineapigs and 12 rabbits. From titration experiments it appeared that following fatal cerebral infection the virus is ten times more prevalent in rabbit brain than in guineapig brain, and that the guineapig is about 100 times more resistant to subcutaneous dosage than is the rabbit. "Over and above the working of these two factors, guineapig passage appears to achieve some actual attenuation of virus when tested by subcutaneous inoculation into guineapigs."

According to Remlinger and Bailly 10 the virus of the pseudorabies of Aujeszky when injected into the tissues of a receptive animal does not suffer any "eolipse" (Tropical Diseases Bulletin, Vol. 27, p. 252). It multiplies in situ until it passes into the blood and from thence into the central nervous system. Here it multi-

Shope (Richard E.). Modification of the Pathogenicity of Pseudcrahies Virus by Arimal Passage. J. Experim Med. 1933. June 1, Vol. 57. No. 6, pp. 925-931.

<sup>10</sup> Remlinger (P.) and Bally (J.) Le siege du virus dans la maladie d'Aujerzky experimentale. C. R. Soc. Biel. 1983, Vol. 113, No. 18, pp. 125-126.

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plies abundantly and may be used for subpassage. The points to be noticed are that the Aujeszky virus travels to the central nervous system by way of the blood and lymphatics, and not along the nerves. In certain cases an infected animal may die before the virus has reached the centres. The duration of the illness is so short (a few hours) that the phenomenon of autosterilization does not occur. Nothing is known regarding the localization of the virus in the brain. After the central nervous system has become infective, the virus may pass centrifugally along the peripheral nerves, but death often occurs before this takes place. It will be seen from the above that two stages occur: a blood phase during which the animal may die, and then a nerve phase. It is not surprising that various organs are found to be more infective than is the case in rabies. The disease has been reproduced from inoculations of liver pulp, of spleen, kidney, testicle, suprarenals, bone marrow, etc. "Special mention ought to be made of the presence of the virus in the lung, as was found by Shope in 'mad-itch' which appears to be a form of the disease of Aujeszky." Saliva, bile, urine, and faeces on the other hand failed to infect rabbits and guineapigs. Transmission by biting has always been unsuccessful.

A further study of the virus of mal de caderas has been made by Costa <sup>11</sup>. It will be remembered (this Bulletin, Vol. 29, page 189) that Remlinger and Bailly, from extended experiments with a virus supplied by Rosenbusch, concluded that it was a rabies virus. Costa comes to the same conclusion, but adds that it is a strain of rabies virus with peculiar modifications. Its clinical manifestations in herbivora are not the same as those of rabies virus, and transmission to man appears to be very unusual. A sero-vaccine prepared, after the manner of Fermi, from a strain of mal de caderas virus which had been fixed by subpassage, afforded adequate protection against the disease, but a similar vaccine prepared directly from a cow which was killed in the paralytic phase of mal de caderas was ineffective. The fixed virus vaccine consisted of 3 parts of a 30 per cent. brain emulsion added to 1 part of immune serum. It is stated that since prophylactic treatment with this vaccine 'the epizootic has totally disappeared'.

Zibordi <sup>12</sup> has failed on three occasions to transmit fixed virus through the pulp of the canine tooth of the dog. The injections were made through an opening which had previously been made with a trephine. A similar experiment on two dogs with street virus also failed. Rabies did not develop in any of the animals

<sup>&</sup>lt;sup>11</sup> Costa (M. Humberto.) Contribucion al estudio del mai de caderas de los hovinos. II. Estudio experimental del virusy la vacuna.—Rev. Higiène y San. Pecuarias. 1933. Jan.—Feb. Vol. 23, No. 1-2, pp. 5-24. [16 Refs.]

<sup>&</sup>lt;sup>12</sup> Ziberdi (I). Studi sperimentali sulla trasmissione della rabbia.—Giorn. di Batteriol. e Immunol. 1932. Dec. Vol. 9, No. 6, pp. 929-940. 20 refs.

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inoculated by this route. That the viruses were potent was proved by control experiments using various routes.

#### II. SYMPTOMATOLOGY.

A case of rabies with an incubation period of 14 months is described by Hajare. 13

The clinical features of two cases of rabies are reported from Philadelphia, U. S. A., by Reisman, Fox, Alpers and Cooper 14. In one of the cases an autopsy was performed, and the microscopic appearances in the brain and cord are described in detail. Neither case presented any unusual features.

### III. DIAGNOSIS.

Serebrennaja and Pugatsch <sup>15</sup> recount their experiences in the diagnosis of rabies. During six years they have examined 1,096 brains of suspected animals. Of the three methods of staining employed, those of Stutzer, Muromzew and Turewitsch, they have found the last to be the most exact. The composition of this stain given as haematoxylin, acid fuchsin and pieric acid, without further details. From their results they conclude that Negri bodies are living growing bodies with a definite life cycle.

# IV. PATHOLOGY.

It will be remembered that Marie <sup>16</sup> obtained contradictory results when rabies virus was inoculated intramuscularly, intravenously or subscutaneously, after the reticulo-endothelial system had been blocked with Indian ink. He has now repeated these experiments with the difference that the infecting dose was introduced into the peritoneal cavity. The experimental details will be published later; the conclusions are stated to be as follows: (1) That even large doses of fixed virus do not infect when introduced into the peritoneal cavity is well known (2) If, however, Indian ink emulsion is previously injected, infection occurs in

<sup>13</sup> Hajare (S.S.). A Case of Rabies with an unusually long Incubation Period.—Indian Med. Gaz. 1933. Apr. Vol. 68. No. 4, p. 212.

<sup>14</sup> Riesman (David), Fox (W. W.), Alpers (B. J.) and Cooper (David A.) Hydrophobia. Report of Two Fatal Cases with Pathologic Studies in One.—Arch. Intern. Med. 1933. May, Vol. 51, No. 5. pp. 643-655. With 8 figs.

<sup>&</sup>lt;sup>16</sup> Serebrennaja (A.) and Pugatsch (E.) Ueber die Rolle der Negrikorper bei der Diagnosestellung und Actiologie der Tollwut.—Zent. f. Bakt. I. Abt. Orig. 1933. Feb. 23. Vol. 127, No. 7-8, pp. 417-426.

Marie (A. O.) De L'infection rabique par la voie peritoneale.—C. R. Soc. Biol. 1932, Dec. 16, Vol. III, No. 39, pp. 869-870.

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guineapigs with an incubation period of from 9-20 days. In two sets of six animals, only one of the first set and two of the second failed to contract the disease. (3) A suspension of 'Deinse' (2 cc. phosphate disodique 5 per cent., with 0.5 cc. chloride of calcium 5 per cent.) gave a similar result. (4) Guineapigs which survived after intraperitoneal injection of fixed virus (without previous treatment by Indian ink) were found to be not immune to intraocular inoculation.

Havens and Mayfield 17 continue their study of the antigenic properties of rabies virus (Vol. 29, p. 601). In the earlier communication it was found that fixed virus immune serum failed to cause flocculation of 1 out of 16 strains of street virus. The authors have now found three other strains which were not agglutinable. The antigenic composition of these inagglutinable strains are investigated in the present communication, using fixed virus as a basis for com-Nine strains (including the 4 non-agglutivable strains and one of fixed virus) and nine homologous immune sera, were set up against each other. The fixed virus was agglutinated by all the sera in approximately the similar degree, suggesting that all the strains possessed a common factor. 'That they possessed additional factors in their antigenic composition was shown by the failure of all but one to agglutinate to the titre of the fixed virus'. No two of the strains appeared to be identical. Cross absorption tests were then carried out. That the absorption was specific was indicated by the tact that normal brain suspensions had no absorptive power. Marked dissimilarities between particular strains were observed, but again there was evidence of the existence of some common factor. Viricidal tests were then carried out and these again disclosed the existence of apparently fundamental differences between the strains. They also indicated, however, that it is the common factor that is most essential in the production of immunity'. The differences observed seem to be due to the presence or absence in whole or in part, of additional antigenic factors.

Greval <sup>18</sup>, <sup>19</sup> describes in detail a method based upon the Wassermann technique, for estimating the degree of complement fixation. He proposes to use this method both for comparisons of sera, and for comparisons of antirabic vaccines as antigens. From experiments, which are not given in detail, it appeared that the antigenic capacity of vaccines improved with keeping, and that rabbit

<sup>&</sup>lt;sup>19</sup> Greval (S. D. S.). The Role of Serology in Rabies.—Indian Med. Gaz. 1932. Dec. Vol. 67. No. 12. pp. 676-678. [13 refs.]



<sup>&</sup>lt;sup>17</sup> Havens (Leon C.) and Mayfield (Catherine R.). Antigenic Properties of the Virus of Rabies. 11. Multiplicity of Strains as shown by Agglutinin Absorption and Neutralization.—*Jl. Infect. Dis.*, 1932. Nov.—Dec. Vol. 51, No. 3, pp. 511-518.

<sup>&</sup>lt;sup>18</sup> Greval (S. D. S.). On Rabies. Complement Fixation in Rabies; the Technique, its Purpose and Associated Considerations,—*Indian Jt. Med. Res.* 1933. Jan. Vol. 20. No. 3. pp. 913-920. [13 refs.]

brain vaccines were less potent as antigens than sheep brain antigens. He uses for complement fixation as antigen, a filtrate of a three months old 5 per cent. carbolized Paris (virus) sheep (brain) vaccine prepared as follows: The brain of a sheep is made into an 8 per cent. emulsion with 1 per cent. carbolic acid in normal saline; this is incubated for 24 hours at 37°C., and then diluted to a 5 per cent. suspension with normal saline. This is kept at room temperature for about 30 days before use.

A case of transmission of fixed virus infection from mother to young is reported by Schneider and McGroarty. A sheep was inoculated subdurally with 0.2 cc. fixed virus. Two days later it gave birth to a lamb. The sheep died of rabies on the sixth day after inoculation having nursed the lamb for four days. Twelve days after birth the lamb showed definite symptoms of rabies. The brain of the lamb was injected subdurally into two rabbits; both rabbits died of rabies in seven days.

#### V. METHODS OF TREATMENT AND STATISTICS.

The investigation on the relative immunizing potencies of etherized and carbolized vaccines undertaken by Cunningham and his co-workers in accordance with the resolution of the International Rabies Conference (Paris 1927) has been frequently referred to in these reviews. (Vol. 24, p. 229; Vol. 25, p. 190; Vol. 26, p. 219; Vol. 27, p. 742.) It will be remembered that the results both of treatment of human beings and of experiments on rabbits and monkeys were best in the case of those treated by Alivisatos' method, less good when Hempt's vaccine was used, and poorest with carbolized vaccines. In those experiments, however, there was great variation in the dosage of nerve substance, the quantities being (for human beings) 6 to 8, 4, and 0.7 gm. respectively. It was realized that until the three methods were contrasted in uniform dosage no definite conclusion as to relative efficiency could be drawn. The present communication<sup>21</sup> by Cunningham, Malone and Craighead deals with certain aspects of this problem. Carbolized vaccines were given to 4,474 persons, and ether carbolized vaccines to 4.513 according to the alternate case system, and as a check 1,598 persons of the type of Hempt's Class III were treated by Alivisatos' method. In each case the

Schneider (J. E.) & McGrearty (B. J.). Transmission of Experimental Rabies from Mother to Young.—Jl. Amer. Vet. Med. Assoc. 1933. Apr. Vol. 82. N. S. Vol. 35. No. 4. P. 627.

<sup>22</sup> Cunningham (I.), Malone (R. H.) & Craighead (A. C.). An Investigation into the Value of an Etherized Vaccine in the Prophylactic Treatment of Rabies. Part VIII. The Comparative Immunizing Value of Carbolized, Etherized Carbolized and Etherized Vaccines Tested under Identical Conditions of Desage and Administration.—Indian Med. Res. Memoirs, Supplementary Series to Indian II. Med. Res. 1833. Jan. Memoir No. 26. Pp. VII and 144 with one chart.

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vaccine was a 5 per cent, emulsion of brain substance. The figures are given in detail, and the results have been examined for significant differences by McKendrick (Appendix B). The general conclusion is arrived at that 5 per cent. carbolized and 5 per cent. Alivisatos' vaccines are similar and both superior to 5 per cent. etherized-carbolized vaccine. A large experiment was conducted upon animals. With regard to the method of preparation of the vaccine it appears that 5 per cent. Alivisatos' vaccine is superior to 5 per cent. etherized-carbolized vaccine and to no treatment, and that 5 per cent. carbolized vaccine takes a midposition between Alivisatos' vaccine and etherized-carbolized vaccine, though it is not significantly different from either. As regards strain of virus, Paris fixed virus is superior to Kasauli fixed virus. (These are in brief the general conclusions of an investigation which is an example of thoroughness and of patient labour. That the comparison of methods of treatment is a laborious business will be recognized by all who read this exhaustive memoir of 144 pages. It should be studied not only by those interested in rabies, but by those who are concerned with treatment by vaccines of any sort.)

The vaccines of Fermi and Philipps are compared by Palawandow & Serebrennaja (Odessa). Because of the weak bactericidal power of glycerine and its thick consistence, the preparation of Philipps' vaccine involves a loss of about 30 per cent. of material. Fermi's vaccine requires no dilutions once it is prepared, whereas Philipps' vaccine has to be diluted daily. The vaccine of Fermi is always sterile, whereas about 10 per cent. of Philipps' vaccine has to be discarded on account of bacterial contamination. The immunizing power of Fermi's vaccine is the higher, as also is the rabicidal power produced in the blood. The advantages which are possessed by Fermi's vaccine are probably due to the larger quantity of nerve substance which it contains, and also to the action of the phenol.

A third analytical review by McKendriek <sup>23</sup> of statistics from Pasteur Institutes on the results of antirable treatment undertaken by the Health Section of the League of Nations at the instance of the International Rabies Conference (Paris 1927) has appeared. It deals with the data relating to 69,541 treated persons, and relates mainly to the year 1930.

The figures under review again emphasize the fact that the non European is at a much higher degree of risk than the European. The mortalities for the two race groups are in the ratio of 7.8 to 1.

<sup>&</sup>lt;sup>22</sup>. Palawandow (Haidar) & Serebrenzaja (A. J.). Vergleichende experimentelle Untersuchungen ueber die Methoden von Fermi und Philipps.—Zischr, f. Immunitatsf. u. Experim. Therap. 193<sup>3</sup>. Vol. 78. No. 3/4. Pp. 217-230. (13 refs.)

<sup>&</sup>lt;sup>23</sup>. McKendrick (A. G.). A Third Analytical Review of Reports from Pasteur Institutes on the Results of Anti-Rabies Treatment.— Quant. Eull. Health Organisation, League of Nations. Geneva. 1932. Dec. Vol. I. No. 4. Pp. 725-755.

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No evidence has been found which suggests the existence of a superiority of any one method of treatment over any other, or, more generally, there is no evidence of the existence of a superiority of treatment by live vaccines over treatment by killed vaccines. With regard to this point the reader is referred to the following table which comprises such figures as it was possible to extract from the three reviews relating to persons of a single race type (European) who were bitten by animals which were certified to be rabid (Categories A and B).

	Number	Deat	hs	Mortality
Treatment	treated	Observed	Calculated*	per cent.
Cords .	1,784	5	3.4	0.28
Dilutions	4,731	7	9.1	0.12
Killed phenol	2,920	4	5*6	0.14
Live phenol	526	. 2	1.0	0.38
Hented	6,685	16	12.0	0.24
Ether killed	5,098	8	9.8	0-16
Mixed .	5,338	10	10.3	0.19
Ether not killed	1,978	4	3.8	0.20
	29,060	56		0.19

\* From the average mortality.

The probability that equal or greater differences than those observed would be likely to occur in a population which was admittedly homogeneous is found to be 0.8 so that there is no evidence of heterogeneity in the statistics gathered over the whole period. If the mixed group and those treated by ether (not killed) vaccines be combined, as seems reasonable since in both groups the treatment of severe cases is by the method of Alivisatos, the figures are as follows: number treated 7,316: observed deaths 14: calculated deaths 14:1: mortality per cent. 0.19.

From the figures submitted it would appear that liability to accident varies significantly according to the method of treatment employed. The relative incidence from the figures collected since the inception of these reviews is given in Section XII. It appears that the liability to accident from live vaccines is greater than the liability from killed, and that the liability from killed vaccines is greater than the liability from heated.

The table referred to deals with the combined statistics covered by the three reviews and is as follows:—

	Treatment.	Number treated.	Accidents.	Proportion.	Percentage.
Cords Dilution Killed phenol Live phenol Heated Ether killed Mixed Ether not kill		33,751 14,649 59,911 1,117 22,716 22,038 13,088 3,634	17 4 5 0 0 3 4 0	1 in 1,985 1 in 3,662 1 in 11,982  1 in 7,346 1 in 3,272 	0°051 0°030 0°0855 < 0°09 < 0°04 0°04 0°031 < 0°028

If the last two groups be combined the figures are serially 16, 722; 4; 1 in 4,180 and 0.024 per cent.

In the annual report of the Pasteur Institute at Kasauli (India) Shortt<sup>24</sup> reports that as a result of the series of experiments carried out by Cunningham and his co-workers, the vaccine now employed is, as before that of Semple, but that the dosage has been largely increased in the case of the severely bitten. The dosage for the slightly bitten is 1,400 mgm. during 7 days, whilst for the severely bitten it has been increased to a maximum of 8,500 mgm. in 15 days. The figures are still experimental in the sense that Paris and Kasauli strains of fixed virus are compared, as are also vaccine from the sheep and from the rabbit. The combined results are as follows:—

			,	Cases.	Deaths.	Mortality per cent.
Europeans treated at Institute Non-Europeans treated at Institute Europeans treated outside Non-Europeans treated outside	•			314 7,389 801 2,634	0 95 0 8	0 1-29 0 0

One rather doubtful case of post-vaccinial paralysis is reported. The percentage of health returns received after six months from the termination of treatment for those treated at the institute is above 72 per cent., and for those treated at outcentres it is above 63 per cent.

<sup>&</sup>lt;sup>24</sup> Kasauli, Pasteur Institute of India. The Thirty-First Annual Report of the Director of the Institute for 1931. Part II, (Scientific). Shortt (H. E.), Director, 46 pages. Delhi Government Printers.

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At Coonoor<sup>25</sup> the dosage of carbolized vaccine has also been increased; in this case the variation is between 700 and 3,500 mgm. administered during 14 days. The results published by Iyengar, are as follows:—

							Cases.	Deaths.	Mortality per cent.
Europeans at Institute . Non-Europeans at Institute Europeans outside .		*	4 *	4	9 -		40 462 236	0 7	0 1:51 0:75
Non-Europeans outside	:		:			٠	7,790	58	0.74

No cases of post-vaccinial paralysis have occurred. The percentage of health returns received six months after the expiration of treatment is above 94 per cent.

The method of Semple is employed at the 'Instituto Vital Brasil'. The number treated is 544, and no deaths from rabies nor cases of post-vaccinial paralysis have been observed. Brasil and Leal<sup>26</sup> discuss the treatment of rabies generally.

Viala<sup>27</sup> reports that during the year 1932, 561 persons were treated at the Pasteur Institute, Paris, and that none of these developed rabies. No post-vaccinial paralysis were observed.

#### VI. RABIES IN ANIMALS.

From experiments on 45 frogs (Runa temporaria) Battaglino<sup>28</sup> confirms the observation of Remlinger and Bailly that the frog is refractory to rabic infection. The frogs were killed at intervals and their brains tested for virulence. In frogs which were in a state of lethargy, virulence was retained for a period of about 32 days, whereas when the frogs were active virulence was lost after about 3 days. The tests for virulence were carried out on guineapigs, which were more susceptible than rabbits. The virus recovered from the frog's brain had not become attenuated.

Five more cases of rabies in the mongoose are reported by Greval<sup>29</sup> from India.

<sup>25</sup> Coonoor, Pasteur Institute of Southern India: Twenty-Fifth Report of the Director of the Institute for 1931. Iyengar (K. R. K.), Director. 69 pages. Madras: Madras Publishing House.

<sup>&</sup>lt;sup>26</sup> Brasil (Vital), Jr. and Leal (A. Estillac). Vaccinacao anti-rabica pelo methodo de Semple,-Brasil-Medico, 1932, November 5, Vol. 46. No. 45, pages 931-933.

<sup>&</sup>lt;sup>27</sup> Viala (Jules). Les Vaccinations antirabiques a l'Institute Pasteur en 1932. Ann. Inst. Pasteur, 1933, May, Vol. 50, No. 5, pages 745-748.

<sup>28</sup> Battaglino (Giuseppe). Contributo allo studio Del virus rabido nella rana. Giorn di Batteriol. a Immunol. 1932, December, Vol. 9, No. 6, pages 961-971. English summary (5 lines).

<sup>&</sup>lt;sup>39</sup> Greval (S. D. S.). Rabies in the Mongoose. Further Observations. Indian Medical Gazette. 1933, Jan., Vol. 58, No. 1, pp. 20-23.

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The statistics of post-infectional antirabic treatment of domestic animals in Hungary during the last thirty years are analysed by Aujeszky<sup>30</sup>. In all 19,979 domestics animals have received treatment. The method employed is a modification of the dilution method of Hogyes (eight inoculations during 6 to 9 days). Information regarding the fate of the animal was available in 19,583 cases. Of these 347 died during the course of treatment or during the succeeding 14 days; of the remaining 19,236, 130 developed rabies (0.67 per cent.). Three cases of paralytic accident, none of which were fatal, were observed. Data are also given of the incubation periods observed in the different animals.

Statistics of preventive inoculation of animals furnished by Balozet<sup>31</sup> from Tunis, show that of 482 dogs vaccinated prophylactically during the period 1st October 1931 to 31st December 1932, three developed rabies. These three had been bitten by a rabid dog a month or two before vaccination, and so cannot be considered as failures or prophylactic inoculation. The effect of the prophylactic measures is demonstrated by the following figures from the town of Bizerta. In 1931, 22 cases of rabies occurred. In the first quarter of 1932, 20 cases were reported. In the 2nd quarter, that is 9 to 15 months after the administration of the vaccine, only one case of rabies was observed.

An 'antirabic week' has been instituted in Japan<sup>32</sup> for the control of rabies in districts where the disease is present. During this period all dogs are registered,

		301	Year	- 7		2.1	No. of rabid dogs.	No. of dogs inoculated.	No. of persons bitten.	No. of deaths from rabies.
1921 .			-							54
1922	-	•	- •	- *	•		**	•••	•••	79
1923 .	2 1	- 50	•		•		2,644	116,050		174
924 .			•		• 1		3,205	194,177	X. X.	23
925 .						•	3,038	255,097		143
1926 .		. 1					1,799	234,680		80
1927 .	Ť.	1					986	200,032	1,446	30
1928 ,	0.7	,					434	225,636	1,122	2
929 .		-					172	145,653	5*6	1
930 .	1		× -	3	200		65	128,753	267	1
931 .	0			- 1	22. 30		44	1.20,100	65	- 1
932 .			1 3				63		09	

<sup>\*</sup> The figure between the 5 and 6 is illegible.

<sup>&</sup>lt;sup>20</sup> Aujeszky (A.). Dreissig Jahre der Postinfektionellen Wutschutzimpfung der Haustiere in Ungarn. Deut. Tierarzt. Woch. 1933, Apr. 8, Vol. 41, No. 14, pp. 209-210.

<sup>&</sup>lt;sup>31</sup> Balozet (L.) La vaccination antirabique des animaux en Tunisie du ler octobre 1931 au 31 decembre 1932. Arch. Inst. Pasteur de Tunis. 1933, Apr., Vol. 21, No. 3, pp. 538—543.

<sup>32</sup> Journal of the Public Health Association of Japan, 1933, Apr. Vol. 9, No. 4, pp. 2-3. Measures taken in Japan for the Prevention of Rabies.

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unclaimed dogs are destroyed, preventive inoculation is given, etc. The results appear in the table (p. 93).

## VII. POST-VACCINIAL PARALYSES.

Three cases of post-vaccinial paralysis amongst persons treated by Semple's carbolized vaccine are described by Stuart and Krikorian 33. They occurred within the period April 28th to July 29th 1932, and two had a fatal termination. The occurrence of such accidents at Jerusalem has been as follows:—The first occurred after 4,580 bitten persons had been treated, the second after 5,409, and the fifth after 9,950. Again, 'while the period 1923-27 was entirely free from accident, the period 1928-32 showed 5 cases of post-treatment paralyses'. Of the five, three showed Landry's syndrome and ended fatally; the other two were cases of dorsomyelitis and have recovered. "A parallel is found in the statistics of the Vienna Institute: whereas between 1894 and 1914, 7,632 bitten persons were treated without accident, between 1915 and 1923, 6,764 treatments yielded 39 cases (5.7 per thousand)." These periodic increases cannot be ascribed to intensification of treatment or be explained by the numbers of passages to which the fixed virus used has been submitted (Bujwid's theory). They lend support to the view that the inci-The histopathological appearances were those of dence shows periodic increases. varying degrees of degenerative change in the nerve cells, with considerable perivascular congestion. Perivascular demyelination and "cuffing" were absent. Negri bodies were found. "A cytotoxin, rather than a virus theory of origin, is again advanced. Thus in the basic nerve substance of all anti-rabic vaccines there seems to exist some deleterious component which, though adversely affected by various physical and chemical agencies, is still capable, in peculiarly susceptible individuals, of producing neuroparalytic disorders."

# VIII. MISCELLANEOUS.

From an experiment carried out on 12 guineapigs, three treated with fixed virus brain, three with normal brain, and three controls, Dolfini34 comes to the conclusion that anti-rabic treatment (method of Puntoni) has no influence on the occurrence of characteristics of anaphylactic shock induced by horse serum. As anti-rabic vaccine is rich in lipoids, this result is considered to be of importance in relation to the view held by many that anaphylactic shock is inhibited by the parenteral injection of lipoids.

<sup>38</sup> Stuart (G) & Krikarian (K.S.). Neuroparalytic Accident's Complicating Antirabic Treatment.

Brit. Med. Jl. 1933. Mar. 25, pp. 501-504. [21 refs.]

34 Dolfini (G.). Puo la vaccinazione antirabica medificare uno stato di sensibilizzazione allergios ?-Policlinico. Sez. Prat. 1933. Feb. 20. Vol. 40. No. 8. Pp. 285-287. (20 refs.)

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In the second part of a memoir entitled "Studies in Rabies", Remlinger and Bailly <sup>15</sup> have collected a number of recent papers. In the first and second sections they describe the treatment of dogs and other animals by etherized virus, and give the results of their experience in Morocco. (This Bulletin, Vol. 30, p. 137, and earlier papers). In the second they describe their experiments with the Trinidad virus (Vol. 29, p. 596). The third section deals with the effects of alcoholism during immunization (Vol. 30, p. 143). The fourth on phylazis deals with the action of sparteine (Vol. 30, p. 140). The fifth on the persistence of rabies virus in the brain of the tortoise has already been reviewed (Vol. 29, p. 605, and Vol. 30, p. 141). The sixth is on the influence of cachexia on the pathogenesis of rabies (Vol. 29, p. 607). The seventh discusses the influence of anaesthesia on the incubation period or rabies (Vol. 29, p. 607). The eighth and last is on the passage of rabies virus through the milk (Vol. 30, p. 137). The results of these researches are thus already in the hands of the reader.

<sup>35</sup> Remlinger (P.) & Bailly (J.). Etudes sur la rage (deuxeme memoire). Ann. Inst. Pasteur. 1932. Dec. Vol. 49. No. 6. Pp. 665-735. (Refs. in footnotes.)

# FURTHER NOTE ON FOOT-AND-MOUTH DISEASE IN SOUTHERN RHODESIA AND AN EMERGENCY METHOD OF INOCULATION

BY

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[ Reprinted from Transactions of the Royal Society of Tropical Medicine and Hygiene, Vol. XXVII, No. 1.]

In December, 1931, an article was sent to this Society for publication describing certain unusual teatures presented by foot-and-mouth disease in Southern Rhodesia. A method of emergency inoculation based upon these peculiarities was suggested, with a view to setting up a mild form of the disease and establishing herd immunity. This article being too long, an epitome only appeared in these *Transactions* (1932), Vol. XXVI, No. 1 (June), p. 89. It stated that at the time the report was compiled some 20,000 to 30,000 cattle had been inoculated by the method described, and a further and fuller report, embodying the observations of field officers in charge of the work and giving details of practical and scientific interest, was promised.

The special features of the disease as it occurred in Southern Rhodesia, in the laboratory and in the field, were briefly as follows :- In the majority of cattle the infection was extremely mild, "occult" cases characterised by thermal reaction only without "open "lesions, predominating. The blood of cattle suffering from such an "occult" reaction was found to be virulent even from the first rise of temperature. Under Rhodesian conditions, also, infection was only transmitted with difficulty, close head-to-head contact being necessary. In the laboratory, attempts to infect by intra-oral and subcutaneous inoculation failed, but intra-nasal injections were almost invariably successful. It was also found that when virus of low virulence was passed from animal to animal in low condition the mildness of reaction persisted, but that a single passage through a fat animal would result in an exaltation of virulence and the appearance of typical lesions. The viability of the virus outside the animal body was extremely brief even under laboratory conditions, and at first it was believed that it was equally vulnerable in vitro. This, however, was found to be incorrect and, when a preservative of appropriate composition and pH had been found, blood-virus and infected epithelium were sent to England where they arrived virulent and maintained their infectivity for several months.

The slow spread of infection under natural conditions, the indefinite nature of the symptoms, the vast and inaccessible areas in which the disease occurred, and

the innumerable native-owned cattle to be controlled, rendered the task of the Veterinary Department extremely difficult, and some emergency method of dealing with the disease had to be devised. Fortunately the exceptional features described suggested to the writer three methods of dealing with the disease, and these were boldly and successfully adopted; with the result that twenty months after the appearance of the disease some 185,388 cattle having been inoculated, the disease has been completely eliminated from the Colony—a notable achievement of which the veterinary profession in that country has every reason to be proud.

The three methods advocated were :-

Method 1:—The issue of standardised virus from the laboratory to be used by officers in the field.

Method 2:- The preparation of virus by veterinary officers in the field, in accordance with the following directions:—From a naturally infected herd some dozen hardy animals to be selected and kept under observation. These to be young animals of "native" or Africander type, not too fat, thin, or suffering from any "open" lesion. Their temperatures to be taken daily. On an elevation of temperature indicative of foot-and-mouth disease, blood to be taken and injected into the noses of the others. When these in turn reacted their blood also to be drawn into separate bottles containing glycerine and citrate-saline solution to make equal parts of each. The bottle containing the blood-virus mixture to be kept in a cool dark place in order that piroplasms, trypanosomes and other contaminants might die out. The donors to be watched and the blood from any reacting severely to be discarded. The supplies from suitable reactors to be pooled and 21 c.c. of the pooled mixture to be injected into each nostril of the animal to be inoculated. During the following fortnight the inoculated animals were not to be driven or exposed to any severe condition and were to be regarded as infective to healthy animals with which they came in contact.

It may be mentioned that as much as 1,500 c.c. of infective blood could be taken from each bleeder and this mixed with preservative provided 4,500 c.cm. of liquid containing virus.

Method 3:—This was based upon the observation that infection could be transmitted by close head-to-head contact. It was suggested that to hasten infection sick and healthy animals should be herded together in closest contact in kraals for several days, and that, if possible, rock-salt or maize-meal should be put down to encourage head-to-head contact.

The Report of the Chief Veterinary Surgeon for Southern Rhodesia for the year 1932 has now been published and contains interesting information concerning the application of these methods which have resulted in the elimination of foot-and-

mouth disease from Southern Rhodesia. Reports from his District Veterinary Officers are included which show that the original directions were in some cases slightly modified but that the general principles laid down were adhered to, one or other of the methods being adopted. Generally speaking, the idea was to "burn the disease out" instead of allowing it to smoulder indefinitely, and to establish belts of immune cattle between infected and clean areas. The Chief Veterinary Surgeon states, "It was indicated in my report for 1931 that two of the methods recommended by the Director of Veterinary Research, Mr. Ll. E. W. Bevan, for the rapid spread of infection in areas likely to become infected were adopted. The direct contact method, that is, the introduction of diseased animals was carried out on a large scale in the Victoria area with very satisfactory results. In all other areas the intra-nasal method of inoculation was practised. The total number of cattle inoculated during the year was 185,388. The results were extraordinarily satisfactory, the reactions being invariably well marked, practically 100 per cent., excluding calves, and all signs of active infection had generally disappeared in less than four weeks from the date of inoculation. In two instances only was there any variation from the normal reactions; in these, fresh cases of disease continued to appear up to thirty days from the date of inoculation. Inoculation, of course, cannot be successful unless a cattle-free belt is established between the area in which it is being carried out and the surrounding clean country. The method usually adopted was to concentrate the cattle for inoculation and, where feasible, move cattle not in immediate contact with infection outwards, thus creating a cattle-free belt, both sides of which were strongly guarded." Since the elimination of the disease from Rhodesia it has appeared in the Bechuanaland Protectorate, where it is being dealt with by the intra-nasal method of inoculation slightly modified to local conditions. According to the latest reports some 85,000 cattle have been treated.

In the course of this unique experiment on an enormous scale, several interesting observations were made by officers in the field. With regard to the duration of immunity resulting from infection with the Rhodesian virus the Chief Veterinary Surgeon states, "There is no doubt that it gives a complete immunity for a considerable period. This is demonstrated by the inoculation with proved virulent blood of 266 head of transport oxen at the Nuanetsi Ranch which had been infected about fourteen months previously. Not a single beast showed the slightest sign of a reaction to the inoculation."

One officer presents evidence which indicates that calves born of cows shortly after recovery from natural infection are immune. Concerning methods of dissemination of the disease in Rhodesia, experience has gone to prove the accuracy of the earlier theories. The Chief Veterinary Surgeon writes:—" It can be stated with

confidence that the great majority of outbreaks during the year were the result of contact with diseased animals...... There is no doubt that game, especially some of the larger species, contract the disease readily, but there is no information as to the course it pursues when a herd becomes infected...... At the same time it is evident that game cannot be a frequent cause of the dissemination of infection, otherwise no measures would have prevented the greater part of the Colony becoming involved."

Although numerous opportunities must have occurred to ascertain whether recovered animals become "carriers" of infection, some 795,000 head of cattle having contracted the disease naturally and a further 185,000 as the result of inoculation, no instance of such a case has been recorded.

It is of interest to note that the Rhodesian virus was sent by the writer in January, 1932, to the Weybridge Laboratory to be "typed". The first passage through English cattle resulted in a severe reaction with characteristic lesions. Also the resulting infection proved to be readily transmissible from animal to animal. The viability of the virus outside the animal body and in body tissues and on different substrata, appeared to be approximately the same as for European viruses. In other words, with a single passage, the Rhodesian virus lost its local characteristics. On the other hand, in Rhodesia the virus dose not appear to have undergone any change or mutation in cattle, otherwise the inoculation process would not have proved so successful.

## **ABSTRACTS**

Therapeutique generale de piroplasmoses. (Treatment of piroplasmosis.) Donatien, A., and Lestoguard, F. Rev. Vet. 85, 417-433 (1933).

In this comprehensive article, the authors review, under appropriate headings (as indicated below), the present position in regard to the prospects of cure and prevention of the various forms of piroplasmosis affecting cattle and solipeds, the parasites considered by them comprising Piroplasma bigeminum (cosmopolitan), Babesiella bovis (European), Babesiella berbera (North African), Babesiella argentina (South American), Anaplasma marginale (cosmopolitan), Theileria dispar (North African and Asiatic), Theileria parva (South African), Piroplasma cabalti (cosmopolitan), and Nuttallia equi (cosmopolitan).

#### A. CURATIVE TREATMENT.

#### I. Specific Treatment.

- (1) Trypanblue.—This drug is only effective for true piroplasmosis due to parasites of the subgenus Piroplasma sensu stricto, e.g., P. bigeminum, P. caballi, P. ovis and P. canis. The drug is used intravenously, the doses recommended by the authors being 0.2 grammes for cattle and 0.5 grammes for horses and mules. A single dose is stated to be usually sufficient to effect a cure.
- (2) Piroblue.—This is a combination of Trypanblue with certain bile acids, the latter having the property of reducing the surface tension of liquids and thereby effecting an intimate contact of Trypanblue with the parasites. For cattle, Theiler recommends a dose of 1 to 2 grammes and for horses affected with P. caballi infection, a dose of 2 grammes. Donation and Lestoguard found the drug also effective for nuttalliosis.
- (3) Ichthargen.—This drug would appear to have been used with considerable success in the treatment of babesiellosis (which is not known to occur in India).
- (4) Stoversol.—Used in combination with quinine bromohydrate, it yields favourable results in the treatment of nuttalliesis.
- (5) Salts of Quinine.—This drug has been used with some success in the treatment of anaplasmosis.
  - (6) Hexamethylene Tetramine. Found efficacious for some forms of babesiellosis.
- (7) Antimosan (in 6.3 per cent. solution). In 1931 Velu and his collaborators treated 13 cases of T. dispar infection with this drug and death occurred in 5 of these, but out of a let of 6 animals treated by them in 1932, only 1 died. In an outbreak dealt with by Donatien and Lestoguard themselves, "one-half of the affected animals were treated with Antimosan and the other half left as controls. In the two lots, the total number of deaths amounted to 50 per cent."
- (8) Gonacrine.—Workers in Morocco found a single intravenous dose of 1 gramme of this drug effective for all forms of piroplasmosis both in cattle and sheep. Donatien and Lestoguard found the drug efficacious in babesiellosis, but when employed in doses of 1 to 3 grammes in the treatment of anaplasmosis, it caused only a temporary amelioration of the symptoms and in two instances death occurred as a result of intoxication. The Moroccan workers state that they also treated 21 cases of natural anaplasmosis with this drug and that out of these, 14 survived, those that succumbed having received the drug in an advanced stage of the disease. Donatien and Lestoguard tested the efficacy of this drug in experimental theileriasis and found that although it

suppressed the thermal reaction, it had no effect either on the parasites or on any of the other clinical symptoms.

## II. Symptomatic Treatment.

The procedure recommended under this head is as follows: (1) To induce elimination of toxic products by the administration of diureties; (2) to avoid danger of indigestion, especially in bovines, by the administration of purgatives and by feeding with green fodder; (3) to strengthen the heart by the administration of caffeine or camphor; (4) to impart tone to the system by the repeated injections of glucose; (5) to combat anaemia by blood transfusion; and (6) to take care of the animal during convalescence.

#### B. PROPHYLAXIS.

Under this heading, the authors refer to the desirability of (1) adopting suitable measures against the introduction of affected animals; (2) destroying the transmitting ticks; and (3) premunising all susceptible animals.

By premunising, one contrives to bring about a chronic latent infection in the animal concerned, but as this involves the inoculation of living piroplasms, there is a risk of the occurrence of acute paroxysm following upon the first invasion by the parasites.

In premunising against *P. bigminum* infection, one takes the blood of an animal that had developed a paroxysm of the disease 3 to 4 months previously and inoculates the blood subcutaneously into the animals to be premunised at a dose rate of 5 c.c. If any severe symptoms are produced as a result of the blood inoculation, the animal is injected with 20 c.c. of Trypanblue (presumably of a 1 per cent. solution).

In premunising against anaplasmosis, one injects a healthy animal subcutaneously with 50 c.c. of blood drawn from a bovine in a chronic state of this infection. Six days later, i.e., during the period of incubation (which ranges from 25 to 30 days), the inoculated animal is bled and the blood thus obtained (the "virus vaccine") is injected at a dose rate of 5 c.c. into the animals to be premunised.

There is no method known of attenuating the virus of theileriasis. One therefore selects a stain of parasites from a natural benign case of this disease and keeps the strain alive by passaging through suitable animals, the blood for subinoculation being every time drawn at the moment of acute paroxysm. The blood ("virus vaccine") drawn in this manner is also subinoculated at a dose rate of 10 c.c. into the animals to be premunised.

The three virus vaccines mentioned above may be conserved in vitro for 3 to 4 days (S. K. S.)

# The Life-history of gastrophilus larvae of the horse, and lesions produced by the larvae. Wehr, E. E. Cornell Veterinarian, 23, 254-271 (1933).

The absence of definite information concerning the path of migration of the young larvæ of bot-flies after they have entered the mouth-cavity of the host animal has hitherto made it a matter of some difficulty to determine the right time for the application of control measures against these parasites. The author found that after reaching the mouth, the larvæ of Gastrophilus intestinalis burrow into the mucous membrane of the tongue, cheeks and lips, in which they wander about for several days (which may extend to 3 months or more) and that they later leave these situations and migrate to the stomach by way of the pharynx. It is noteworthy that the larvæ extracted from under the mucous membrane at the base of the tongue were generally much larger than those extracted from the anterior portion of the tongue.

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In regard to G. nasalis, the author cites an interesting observation recorded by Wells [1931] which would seem to indicate that the larvæ of this fly do not borrow into the skin of the throat (where the eggs are, as a rule, deposited), but that they crawl on the surface of the skin and find their way into the mouth between the lips. [S. K. S.]

# The control of camel trypanosomiasis. Bennett, S. C. J. J. Comp. Path. & Ther., XLVI, 67-77 and 174-185 (1933).

The author surveys his work on this problem carried out from 1927-1932 and his experimental data are based on hundreds of camels in which the disease was diagnosed and treated. The species normally responsible for this disease is *Trypanosomo* soudanense (a local name for *Trypanosomo* evansi), which is transmitted by tabanid flies. The number of vectors is very much increased during the rainy season and therefore the disease takes on an epizootic form.

The diagnosis of this disease under field conditions by the cover-slip method was found to miss a large number of infected camels. The formol-gel test gave promising results but on account of certain practical objections and the superiority of the mercuric chloride test the former was abandoned.

The method of applying the mercuric chloride test is to add one drop of suspected scrum to one cubic centimetre of 1/25,000 aqueous chemically pure mercuric chloride solution in a small test tube and mix by gentle movement. The appearance of white precipitate of any density within a few moments indicates infection. For accuracy of results the use of a fresh solution and corpuscle-free scrum is essential.

A single intravenous injection of four grammes of Naganol (Bayer 205) is a curative dose and the author concludes that this drug has no parallel in therapeutic value, and the control of the disease almost entirely depends on the proper administration of this compound.

Immunity following cure is non-existent, although the camels are resistant to re-infection for a short period due to residual traces of Naganol.

Trypanosome "carriers" among domestic animals, such as horses and donkeys, play little part in spreading camel trypanosomiasis.

The routine treatment is generally undertaken towards the end of the "fly" season. This ensures that few camels required a second course of treatment in one year.

Camels are susceptible to all species of testse-borne trypanosomes, and, with the exception of *Tryp. congolense*, the infections appear comparable with those of *Tryp. evansi* type, and a routine system of control would, therefore, be applicable. [B. S.]

# Trichomonads associated with breeding troubles in cattle. McNutt, S. H., Walsh, F. E., and Murray, C. Cornell Vet., XXIII, 160-169, (1933).

The Protozoan parasites known as Trichomenad have long been regarded as causing breeding troubles in cattle. Once the infection is established in a herd, a large number of cows become affected, thereby causing a great loss to breeders. The chief pathological changes found are vaginitis, pyometra, endometritis, early abortion and finally sterility. The infected cows show a distended uterus, containing up to four gallons of pus of a yellowish, watery, and flocculent nature, having a slight odour and acid reaction. The uterine mucosa appears smooth and leather-like, while some parts are roughened yellow and necrotic.

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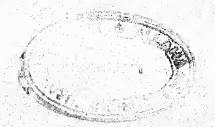
Bacteriological and histological examination gave negative results, but microscopic examination of the exudate showed numerous protezoa with elongated ends and of an average size of  $16 \times 615\mu$ . In old and drying discharges these protozoa take oval and round forms. Microscopic examination of vaginal material after 12 to 18 hours incubation gives better results.

Since the parasttes were also found in the prepuce of bulls it is considered that bulls may act as mechanical carriers of this infection.

When these protozoa were introduced into the pregnant uterus, the authors were able to produce abertion in guinea-pigs, rabbits and in one heifer. [ B. S. ]

The commercial possibilities and limitations of breeding for disease resistance. Cole, L. J. 1933. (Univ. Wisconsin.) *Proc. Amer. Soc. Anim. Prod.* 25th Ann. Meeting, 1932: 271-272.

Natural resistance to many diseases is apparently more prevalent in wild species than in the domesticated races. To incorporate general resistance in a breed it must be found in some members of the breed or be brought in by crossing. The rules of most breed associations effectively prevent introduction of resistance by cross-breeding. There is commonly difficulty in distinguishing natural from acquired immunity, which interferes with the selective process. While genetic resistance may in some cases be conditioned by a single gene pair, it is usually more complex. Resistance to infections is commonly specific, requiring selection for each disease. This greatly limits what can be accomplished. Selection for resistance adds to the characters already being selected for, complicating the breeding programme. Variability (mutation) of the pathogen may upset the results of long selection. Constant selection will be required to retain resistance after it has been attained. The following general principles are offered: (1) Where methods of prophylaxis or treatment are known and readily applicable, they will in general be preferable economically to attempting to breed for resistance. It must be borne in mind, however, that unless the pathogenic agent can be eliminated completely the necessity for control, such, for instance, as by vaccination, will be a continuous one. Under such treatment the stock is likely to come to have less natural resistance rather than more. (2) The genetic method can be much more readily applied to animals which produce a large progeny and in which the value of the individual is relatively small. Progress depends on selection of large numbers and elimination on a large scale. Such elimination is scarcely practicable in the larger animals, for the commercial value of the individual is upt to be too high, and reproduction is so slow that the female breeding stock cannot be maintained if selection is too rigid. (3) From the nature of the case, the number of diseases which might be selected against in any stock is strictly limited. This is another reason for restricting the genetic method to those diseases which cannot readily be brought under control by other means. (4) While in general, as pointed out, the other methods tend away from genetic improvement, it is entirely possible that, at least in certain cases, they may be made to work in harmony and towards the same ond. [From Animal Breeding Abstracts, Vol. I, No. 2, July 1933.]

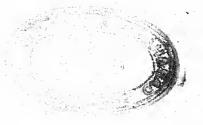


# NOTICE.

# BIHAR AND ORISSA VETERINARY COLLEGE, PATNA.

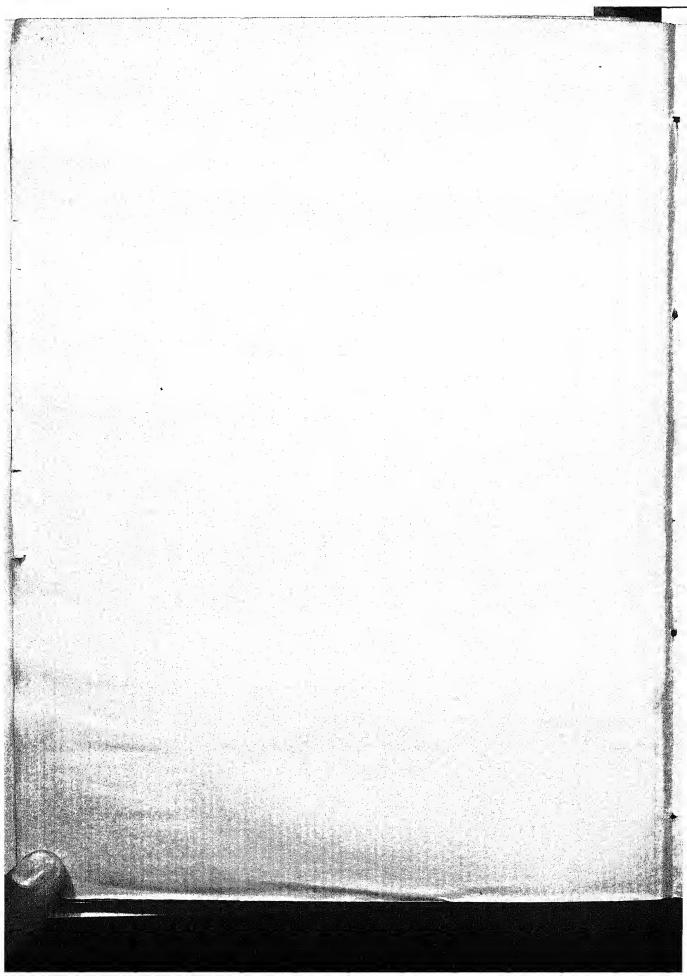
The next session of the Bihar and Orissa Veterinary College will commence from the 1st July 1934.

- 1. Each candidate desiring admission should submit his application on the prescribed form, together with the following certificates in original, so as to reach the Principal on or before the 1st June 1934:—
  - (a) Age and moral character certificate from the Headmaster of the school at which he last read.
  - (b) University certificate or a certificate from the School or University authorities to show that he has passed the Matriculation Examination.
  - (c) Medical Certificate of fitness from an Assistant Surgeon.
  - (d) Letter from his guardian stating that all expenses incurred by his ward during the latter's period of study at the college will be paid.
  - (e) Letter of identification from some well known person stating that the candidate is known to him and that the statements made in the application form are correct.
  - 2. Each Government or District Board stipendiary should, in addition to the above, produce a letter from the Director of Veterinary Services, Bihar and Orissa, or from the Chairman, District Board concerned, to whom he should apply in the first instance, stating that their selection as stipendiary has been approved.
  - 3. Preference will be given to candidates who have passed the I. A. or I. Sc. Examination of a recognised University. A good knowledge of English is essential.
  - 4. Non-stipendiary candidates will have to appear before the Governing Body of the College when called for interview.
  - 5. Fees must be paid in advance according to the scale under rule 8 of the college rules, the initial payment due at the time of admission being Rs. 35-8 only.
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# OBITUARY NOTICE

We deeply regret to record the death, in England, of Mr. Hugh Cooper, M.R.C.V.S., 1st Veterinary Research Officer, Imperial Institute of Veterinary Research, Muktesar, on the 6th December, 1933. In Mr. Cooper's death the Veterinary Profession has lost a most valuable officer and a charming personality cut off in the prime of life, and we offer our deepest sympathy to his bereaved widow. A fuller notice giving some indication of Mr. Cooper's work in this country will appear in a later issue.



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<sup>\* &#</sup>x27;Agriculture and Livestock in India.' 'The Indian Journal of Agricultural Science' and 'The Indian Journal of Veterinary Science and Animal Husbandry' replaced the following publications of the Imperial Department of Agriculture in India with effect from January 1931—

1. The Agricultural Journal of India.

2. The Journal of the Central Bureau for Animal Husbandry and Dairying in India.

4. Bulletins of the Imperial Institute of Agricultural Research, Pusa.

press.

<sup>3.</sup> Memoirs of the Department of Agriculture in India—Botanical, Chemical, Entomological, Bacteriological and Veterinary Series.

<sup>[</sup>A list of the available numbers of these publications can be obtained free on application to the Secretary, Imperial Council of Agricultural Research, Publication Section, Imperial Record Department Building, New Delhi.]

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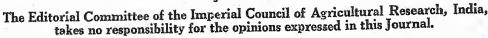
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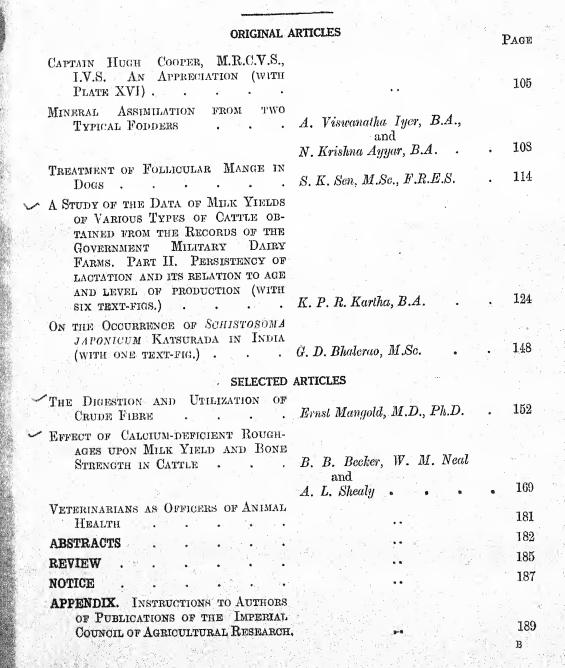
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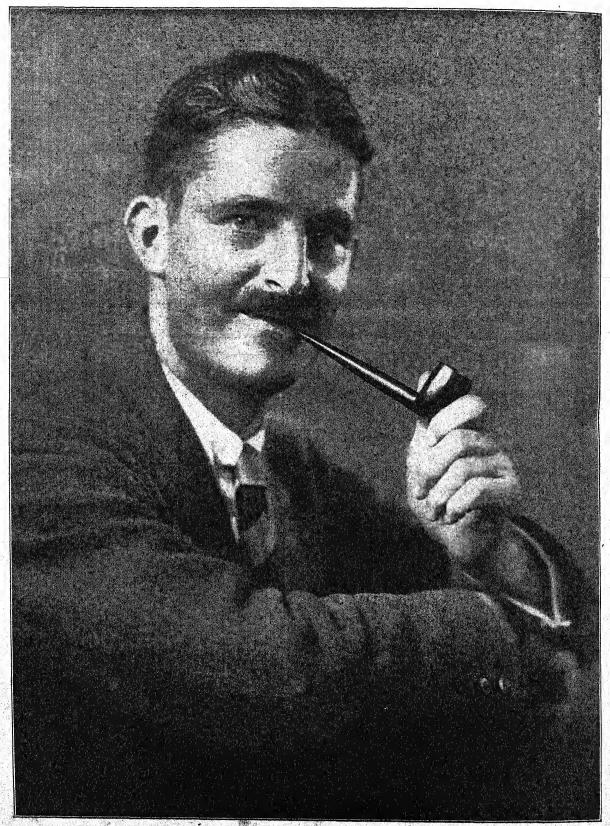
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(VOL. IV, PART II.)

June 1934.







CAPTAIN HUGH COOPER, M.R.C.V.S. (Reproduced with the permission of the Editor, The Veterinary Record.)

# ORIGINAL ARTICLES

CAPTAIN HUGH COOPER, M.R.C.V.S., I.V.S., 1896-1933.

### AN APPRECIATION.

(With Plate XVI.)

It is with the greatest regret that we have to announce the death of Captain Hugh Cooper, Pathologist, Imperial Institute of Veterinary Research, Muktesar, on the 6th December 1933 at Northwood, Middlesex.

The loss of an officer of his calibre and attainments will cause deep sorrow, and his cheering energy and keen application to his work or play, will long be remembered by his colleagues and those associated with him in his work at Muktesar, and also by those members of his profession and others with whom he came in contact throughout India.

We tender our most sincere sympathy to his widow and other relations. As a fitting tribute to his character and courage we feel that we cannot do better than associate ourselves with the appreciation published in the *Veterinary Record*, December 23rd, 1933 issue, which we reprint below:—

"The death of Hugh Cooper, recorded in your issue of last week, cuts short in its prime a promising and useful life. For India, the country in which he had chosen to spend his professional career, this loss must be regarded as a tragic one, for by temperament and bearing he was eminently attuned to that environment, and the large experience which he had been steadily gaining in the problems of animal pathology peculiar to that country would have been of inestimable value when it was duly placed on record in his more mature years from the massive collection of documents and personal knowledge which he had accumulated on the subject during a period of ten years' well-directed industry. The circumstances of his death display the reactions of a gallant nature to the consciousness of a slowly impending doom, the precise outcome of which was clear beyond doubt to his mind by the knowledge gained in his own expert calling.

Born at Hampstead, on September 13th, 1896, the youngest child of the late Mr. C. H. and of Mrs. Cooper, he was educated at Broadoak School, Northwood, and then at Uppingham School, from which he entered the Royal Veterinary College in 1913. He served his pupilage with Mr. F. T. Trewin, of Watford. He qualified in 1917, after winning the Walley Memorial Prize of the R. C. V. S., entered the R. A. V. C. during the War, and saw service in Mesopotamia. After demobilisation, he

went into veterinary practice for a short time, and then returned to the Royal Veterinary College, early in 1920, as a research assistant in the Department of Research in Animal Pathology, soon afterwards becoming Sir John M'Fadyean's Demonstrator in Pathology. He also lectured in protozoology. Towards the end of 1921, he was appointed to the post of Pathologist on the staff of the Imperial Bacteriological Laboratory (now the Imperial Institute of Veterinary Research), Muktesar-Kumaun, U. P., India. He took on his duties there after a short visit for special training to Sir Arnold Theiler's laboratory in South Africa, where he seems to have caught a good deal of that master's zeal for research. At the time of his death, he had spent ten years, as has been said, in actual service, not counting leave periods, at the Muktesar laboratory.

If a selection had to be made of his most important contributions during this period to the advancement of knowledge in veterinary pathology, perhaps the most outstanding would be his extensive and careful survey of the nature and distribution of bovine coccidiosis and piroplasmosis and the resuscitation of latent forms of these infections during intercurrent attacks of rinderpest. There were several other problems that he had gone far to elucidate from among the wealth of material that lay awaiting investigations, notably, for example, pseudo-fowl plague (ranikhet disease), towl Spirochaetosis, glanders, rinderpest in goats, Johne's disease, surra, and bovine theileriasis. It was fully expected that in the normal course of events he would have survived to place on record the masses of evidence which he and other colleagues had gathered round such problems.

His real service to the study and diffusion of knowledge upon animal pathology in India cannot, however, be estimated, even approximately, by his achievements in specialised pieces of large research, important though many of these were. At the Institute, he developed a large and varied routine and advisory pathological side, which was of incalculable benefit to the civil and military veterinary services in the field. His replies to enquiries were invariably characterised by courtesy, helpfulness, precision in diagnosis, and soundness in prognosis. Further, in the classes of training at Muktesar for various grades of veterinary officers in India, including the two years' courses started by the Government of India for the education of Indians in India to the highest standard, he took a prominent part, and his practical teachings will long be remembered by veterinary officers now scattered in responsible positions throughout the sub-continent.

In that isolated hill station his social qualities were an immense asset. Kind, gentle, unfailingly generous and hospitable, he was the friend of everyone: the intimate companion of his European colleagues, he was beloved and trusted by all grades of Indians; these he encouraged at their work, listened to sympathetically in

their difficulties, and fired with enthusiasm in their games. He was a sincere lover of animals.

Soon after his marriage to Miss Janet Beazley in India, in April, 1931, he came home to England on ordinary leave combined with a prolonged period of study leave, which he arranged to spend at Professor Buxton's Research Institute in Animal Pathology at Cambridge, where he was to resume contact with his early friends then on the staff there. He was due to return to his work in India at the beginning of the cold weather season, in the autumn of 1932.

There supervened, however, at this stage in his career, when his future seemed secure and full of bright prospects, one of those unexpected tragedies that seem to fall with special malignity upon those whose minds are fully enlightened as to their fate by the special culture acquired in their avocation and cannot obtain relief in the succour afforded by easy illusion. Characteristically, he faced this position with unwavering courage, betraying till the last no signs of anguish or exhaustion of his ample reserves of sane judgment. Instead, he was coolly solicitous not to inflict his personal sufferings on his friends and relations. At one stage during the course of treatment phenomenal success was achieved; indeed, so remarkable was the improvement in Hugh Cooper's condition that in May, 1933, he was passed fit to return to India, whither he proceeded, accompanied by his wife and young son, overjoyed at regaining a glimpse of the gorgeous Himalayan scenery surrounding his Indian home and getting back to the scenes of his former happy labours. It was not long, however, before the trouble recurred and in July he returned back to England once more. Deeply grateful as he was for all candid advice, he became reconciled to his doom and gallantly met it. The writer now learns that throughout his affliction Cooper kept methodically a daily record of his condition, believing that it might be of some scientific use afterwards. The record is in the custody of the Mount Vernon Hospital. This attitude towards his condition was, it must be felt, a singularly heroic and objective one, but it had no trace of morbidity or callousness about it. His spirit was naturally deeply sensitive and courageous. He who was seen to run unarmed shouting after a panther to rescue his pet bull-terrier from its jaws was not a subject whom familiarity with disease processes and the experimental method had hardened. Rather it seems to have endued him all the more strongly with the calm that pertains to a human caste—that to which he belonged—which holds that in the face of dire personal adversity, certain things, display of subjective emotion among them, are just 'not done'."

# MINERAL ASSIMILATION FROM TWO TYPICAL FODDERS.

RV

### A. VISWANATHA IYER, B.A.,

AND

#### N. KRISHNA AYYAR, B.A.

(From the Animal Nutrition Section, Bangalore.)

(Received for publication on the 25th January 1934.)

The work recorded in this paper is a continuation of experiments published a year ago by Warth, Viswanatha Iyer and Krishna Ayyar [1932]. The references to the literature on the subject and the object of the present enquiry having been set forth fully in the first paper, these matters need not be touched upon here again. The fodders previously tested were *Ragi* straw and mature hay. In the present experiments *Jouar* hay and Rice straw have been tested.

The procedure adopted was exactly the same as that already described. A long period feeding test was carried out with the two fodders, three bullocks being maintained on each. A small amount of concentrate was fed to all animals alike to ensure a sufficiency of protein.

The feeding test was divided into three periods:-

First Period: - The fodder without mineral supplement.

Second Period:—The fodder with supplement of calcium phosphate.

Third Period:—The fodder with calcium phosphate and green food. The last test was intended, as in the previous experiments, to determine whether green food with its accompanying vitamins led to further assimilation. During each period numerous determinations of the mineral balance were made. Variations in the quality of the fodders during the course of the experiment are shown in Table I.

TABLE I.

		Jouar hay		× 1/2 / = 3	Rice straw	
Expt. No.	CaO %	$\mathrm{P}_2\mathrm{O}_5\%$	N %	CaO %	P2O5 %	N %
1	0.248 0.325 0.359 0.344 0.291 0.374 0.570 0.536 0.391	0·301 0·256 0·344 0·256 0·215 0·242 0·232 0·118 0·079	0.651 0.496 0.651 0.514 0.512 0.608 0.688 0.369	0.565 0.557 0.454 0.524 0.483 0.561 0.638	0·139 0·137 0·146 0·155 0·140 0·169 0·204 0·209	0·396 0·419 0·364 0·391 0·368 0·403 0·451 0·539
Total . Average .	3·438 0·382	2·043 0·227	4·883 0·543	5·076 0·564	1:519 0:169	3·872 0·430

It will be noticed that the *Jouar* hay was fairly consistent in composition, except towards the very end of the test when it fell off in quality. The quality of the rice straw improved definitely in the later stages. It is important to compare these figures with values obtained elsewhere. The accompanying table enables us to judge the mineral status of our samples.

Table II.

Showing the average composition of the herbages.

materia salam	CaO	$P_2O_5$	N
Paragraphic spirit of the control of	Per cent.	Per cent.	Per cent.
Great Britain—			
1. Good cultivated pasture	1.004	0.735	2.825
2. Romney Marsh "fatting" field	0.878	1.227	4.38
3. Average Scotch lowland-pastures	0.821	0.875	3.30
4. Average poor Scotch hill-pastures.	0.152	0.420	2.27
South Africa-	10-	* 1	
1. Pasture (average of six samples of hay)	0.135	0.278	-••
2. Green fodder (% on dry matter) Tall Fescue.	0.310	0.120	
3. Sheep's Parsley	0.500	0.160	·
4. Average for whole Season for herbage at Armoeds- vlakte	0.510	0.175	
India			
1. Jouar hay	0.382	0.227	0.543
2. Rice straw	0.564	0-169	0.430

Compared with good English fodders our experimental samples are poor in mineral content. Compared with the South African fodders, the *Jouar* hay is poor in lime, but fairly well provided with phosphoric acid. Rice straw is distinctly poor in phosphoric acid but moderately well provided with lime.

#### EXPERIMENTAL RESULTS.

First Period: - Fodder without mineral supplement.

The mineral balances during the first period are shown in the accompanying table.

First Period—(Fodder without mineral supplement).

		Jou	ar hay	- *		Rice	straw	
		In grams per day	CaO	$P_2O_5$	In grams p	er day	CaO	$P_2O_5$
·		Intake	13.82	19.26	Intake		27.18	11.86
1	$ \cdot $	Exerction	13.22	15.03	Exerction		25.76	10.87
	1	Balance	+0.60	+4.23	Balance		+1.42	+0.99
		Intake	16.76	16.04	Intake		26.10	11.20
2	<b>∤</b>  :	Exerction	12.79	13.49	Exerction		28.90	11.10
	1	Balance	+3.97	+2.55	Balance		<b>—2</b> ·80	+0.10
16. ( 18. ) The	(	Intake	17.47	17.81	Intake		21.45	10.35
3	4	Exerction	14.20	15.24	Excretion		27.34	11.17
	Ų:	Balance	+3.27	+2.57	Balance		5.89	-0.82
		Average	+2.61	+312	Average	* 1 * 1	-2.42	+ 0.09

It should be noted that every figure in this and in subsequent tables is the average intake and outgo determined for six consecutive days with three animals. It is strikingly clear from these results that Jouar hay gives positive assimilation for both lime and phosphoric acid, while rice straw gives negative balances for lime with no assimilation of phosphoric acid. The lime balances are instructive. The rice straw ration, which provides more lime, leads to loss of lime, while the Jouar hay ration, providing much less lime, induces assimilation of lime. These results, coupled with the corresponding phosphoric acid data seem to indicate that lime assimilation is largely dependent upon the phosphoric acid intake. A similar conclusion was reached in the previous paper (loc. cit. page 329). It will be useful at this point to compare the results obtained during the two seasons. The following table shows the average intake and outgo of minerals for the entire series of tests in which the fodders were fed without a mineral supplement.

Table IV.

Results of two years compared.

	CaO	(in grams per	day)	$\mathbf{P_2}0_5$ (in grams per day)			
	Intake	Excretion	Balance	Intake	Excretion	Balance	
Bolarum hay . Rayi straw . Jouar hay Rice straw .	41·17 57·99 16·02 24·91	41·09 55·69 13·40 27·33	+0.08 +2.30 +2.62 -2.42	6.64 12.72 17.70 11.14	9·82 12·67 14·59 11·05	-3·18 +0·05 +3·11 +0·09	

It should be remarked that these figures are the averages obtained from numerous tests with several animals and must be significant.

It appears that if the ration provides a sufficient excess of lime there will be some assimilation even if the phosphoric acid content is low (See results of Ragi straw and Bolarum hay tests published last year). On the other hand, if the lime provided is only moderate in amount it will not be assimilated unless there is an excess of phosphoric acid (Rice straw). Further, the Jouar figures show that even when lime is low, assimilation can occur if excess of phosphoric acid is present.

Second Period: - Fodder with calcium phosphate supplement.

The mineral balances obtained with the supplemented rations are shown in the table given below:—

TABLE V.

Second Period.—(Fodder with calcium phosphate supplement).

<i>ii</i>	Jone	ur hay	1 - 1 - 1 - 2 - 3	Rice straw			
	In grams per day	CaO	$P_2O_5$	In grams per day	CaO	$P_2O_5$	
1	Intake	22·91 18·29 +4·62	20.91 17.81 +3.10	Intake Exerction Balance	$30.93 \\ 29.47 \\ +1.46$	18·92 11·90 +7·02	
2	{ Intake Exerction	19·99 19·18 +0·81	18.75 17.74 +1.01	Intake Exerction Balance	29·32 29·31 + 0·01	$18.32 \\ 16.52 \\ +1.80$	
	Average	+2:72	+2.06	Average	+0.74	- -4.41	

In these experiments the animals received 20 grams calcium phosphate.

First Period—(Fodder without mineral supplement).

TABLE III.

	Jou	ar hay		Rice straw					
Shawer Phrings	In grams per day	CaO .	P <sub>2</sub> O <sub>5</sub>	In grams per o	lay	- CaO	$P_2O_5$		
	Intake	13.82	19:26	Intake .		27:18	-11.80		
	Exerction	13.22	15.03	Exerction .		25.76	10.87		
	Balance	+0.60	+4.23	Balance .	- •	+1.42	+0.99		
	Intake	16.76	16.04	Intake .	•	26.10	11.20		
	Exerction	12.79	13.49	Excretion .		28.90	11.10		
· · · · · · · · · · · · · · · · · · ·	Balance	+3.97	+2.55	Balance .		-2.80	+0.10		
	Intake	17.47	17.81	Intake .	•	21.45	10.35		
.}	Exerction	14.20	15.24	Excretion .		27:34	11-17		
	Balance	+3.27	+2.57	Balance .		—5·89	0.82		
	Average	+2.61	+3·12	Average .		-2.42	+ 0.08		

It should be noted that every figure in this and in subsequent tables is the average intake and outgo determined for six consecutive days with three animals. It is strikingly clear from these results that Jouar hay gives positive assimilation for both lime and phosphoric acid, while rice straw gives negative balances for lime with no assimilation of phosphoric acid. The lime balances are instructive. The rice straw ration, which provides more lime, leads to loss of lime, while the Jouar hay ration, providing much less lime, induces assimilation of lime. These results, coupled with the corresponding phosphoric acid data seem to indicate that lime assimilation is largely dependent upon the phosphoric acid intake. A similar conclusion was reached in the previous paper (loc. cit. page 329). It will be useful at this point to compare the results obtained during the two seasons. The following table shows the average intake and outgo of minerals for the entire series of tests in which the fodders were fed without a mineral supplement.

Table IV.

Results of two years compared.

<b>7</b>	CaO (in grams per day)			P <sub>2</sub> O <sub>5</sub> (in grams per day)			
	Intake	Exerction	Balance	Intake	Excretion	Balance	
Bolarum hay Ragi straw Jouar hay	41·17 57·99 16·02 24·91	41.09 55.69 13.40 27.33	+0.08 +2.30 +2.62 -2.42	6.64 12.72 17.70 11.14	9·82 12·67 14·59 11·05	-3·18 +0·05 +3·11 +0·09	

It should be remarked that these figures are the averages obtained from numerous tests with several animals and must be significant.

It appears that if the ration provides a sufficient excess of lime there will be some assimilation even if the phosphoric acid content is low (See results of Ragi straw and Bolarum hay tests published last year). On the other hand, if the lime provided is only moderate in amount it will not be assimilated unless there is an excess of phosphoric acid (Rice straw). Further, the Jouar figures show that even when lime is low, assimilation can occur if excess of phosphoric acid is present.

Second Period: - Fodder with calcium phosphate supplement.

The mineral balances obtained with the supplemented rations are shown in the table given below:—

TABLE V.

Second Period.—(Fodder with calcium phosphate supplement).

	Jou	ar hay	· · · · · · · · · · · · · · · · · · ·	Rice		
	In grams per day	CaO	$P_2O_5$	In grams per day	CaO	$P_2O_5$
1	Intake	22·91 18·29 4·62	20.91 17.81 +3.10	Intake Excretion Balance	30·93 29·47 +1·46	18.92 11.90 +7.02
2	Intake	19·99 19·18 +0·81	18·75 17·74 +1·01	Intake . Exerction Balance	29·32 29·31 	18:32 16:52 +1:80
	Average	+2.72	+2.06	Average	+0.74	⊹4•4ા

In these experiments the animals received 20 grams calcium phosphate.

It may be said that the supplement has had practically no effect on mineral assimilation with the Jouar ration. Judging by what has been said above, this is perhaps not surprising, for, in the first place, the phosphoric acid balance was positive without the aid of the supplement and therefore added phosphoric acid was not likely to stimulate assimilation to a marked degree; secondly, the lime in the ration, though enhanced by the supplement, is still somewhat low and perhaps insufficient for greatly increased assimilation to take place. With the rice straw ration there has been a marked increase in the phosphoric acid assimilation, but the lime assimilation has not been improved materially. In this respect the result differs from last year's feedings, where addition of calcium phosphate caused a distinct rise in the lime assimilation from the Bolarum hay ration. It is true that Bolarum hay actually provided more lime but this explanation fails when we recollect that lime is readily assimilated from Jouar. It has to be concluded, therefore, that for some reason, lime is not very readily assimilated from rice straw. Possibly this may be due to the absence of appropriate vitamins.

Third Period: - Fodder with mineral and green grass supplements.

The results of the balance experiments carried out during the third period are shown in Table VI.

Table VI.

Third Period.—(Fodder with mineral and green grass supplements).

	Jouar hay			Rice straw			
	In grams per day	CaO	$P_2O_5$	In grams per day	CaO	P2O5	
1	Intake Excretion	27·37 24·00 +3·37	21·97 21·30 + 0·67	Intake Excretion Balance	35·56 32·92 +2·64	21·46 17·92 1-1-3·54	
2	Intake Excretion Balance	35·31 31·11 +4·20	22.66 19.34 +3.32	Intake Excretion Balance	38·23 34·05 4·18	23.61 21.75 1.86	
3	Intake Excretion Balance	29·63 22·17  -7·46	17:39 15:01 +2:38	Intake	38·45 32·49 +5·96	25·13 20·79 4·34	
<b>4</b> , 3, 3, 3, 4, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5,	Intake Excretion Balance	26·23 21·01 +5·22	15·26 13·92 +1·34	Intake Excretion Balance	39.52 41.12 —1.60	23·45 21·99 +1·46	
	Average	+5.08	+1.93	Average	+2.80	+2:80	

In these experiments the animals received 20 grams of calcium phosphate and green fodder.

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It will be recollected that the evidence regarding the influence of green grass was not clear in the last year's tests. In the present tests there is a similar difficulty, but there seems to be some evidence of a positive effect. It is evident, in the first place, that lime assimilation has been definitely enhanced for both rations by the addition of green fodder. But the green fodder has added considerably to the total lime intake and the increased assimilation might be accounted for by this increased intake without having to assume vitamin intervention. Actually the figures show a regular increase in assimilation upto a maximum and this increased assimilation is not due to increased intake. What is the cause of this increase up to a maximum? It may be a vitamin effect.

### CONCLUSIONS AND SUMMARY.

Experiments on mineral assimilation indicate that the assimilation of lime is dependent to a considerable extent upon the intake. There is evidence to show, further, that assimilation of lime is materially influenced by phosphoric acid. A ration with low lime content can induce lime assimilation if phosphoric aid is present in sufficient amount. Finally, the addition of green grass led to increased lime assimilation. This may be accounted for by the extra lime introduced into the ration with the green food, but there is some suggestion of an additional vitamin effect.

Concerning the two fodders tested, Jouar hay, though low in lime content, induced assimilation of lime and phosphoric acid; Rice straw gave negative lime balances and this balance was not satisfactorily rectified by a mineral supplement. There is an indication that lime is not readily assimilated from the type of rice straw used in our tests.

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# TREATMENT OF FOLLICULAR MANGE IN DOGS.\*

 $\mathbf{B}\mathbf{Y}$ 

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Follicular mange occupies an almost unique position among the parasitic diseases of live-stock in that the parasites concerned (Demodex folliculorum) in the production of this condition secure a position in the body of their hosts which is neither sufficiently superficial to be accessable to any of the chemicals ordinarily used in combating other forms of skin affections, nor sufficiently deep-seated to be reached by a chemotherapeutic agent when introduced into the circulatory system of the host. In the matter of the location of the parasites, the disease therefore presents a feature which is not shared by any other parasitic disease, and this fact has led most authors to declare the condition as not being amenable to any of the known forms of treatment. In the case of the canine species in particular, follicular mange is liable to attain a considerable degree of pathogenicity not only by reason of the symptoms specific to the disease itself, but also by reason of the complications which are likely to arise as a result of the introduction of Staphylococcus pyogenes albus along with the invading mites, and in spite of the amazingly large number of remedies that have been tested in the treatment of this condition, the actual prospects of cure cannot be regarded as having substantially improved. As Craig [1923; in Wooldridge, Vol. II tersely puts it, "a large number of methods and applications have been adopted from time to time; a few cases may recover, and the method then enjoys a brief popularity".

Nevertheless, now and again, one comes across reports in periodicals claiming surprisingly good results from the use of remedies which previous experience had shown to be practically valueless in the treatment of the disease, and this would lead one to the conclusion that, at any rate, a proportion of the reported failures to effect a cure may in reality have been due not so much to the inefficacy of the remedy itself as to the method and duration of the application of the remedy. This view would seem to find some support in a statement made by Müller and Glass [1929] in their classical work entitled "Diseases of the Dog", for according to these authors "the nature of the remedy is not as important as the manner or method of appli-

<sup>\*</sup> Being a paper read at the Indian Science Congress, Bombay, January 1934.

cation and, above all, patience and persistence". Similarly, according to Schindelka [cited by Hutyra and Marek, 1926], "many more dogs may be cured by exercising sufficient patience and perseverence than is commonly assumed". It has, therefore seemed to the present writer that the position in regard to the curative treatment of follicular mange is not quite so hopeless as it has generally been made out to be and that there is a genuine field for more intensive trials with some of the remedies already recommended. In what follows it is proposed to do no more than to "take stock" of these remedies, with brief remarks at relevant points as to which of these remedies in the opinion of the present writer holds out the largest promise of success in the treatment of follicular mange in general and in dogs in particular.

A perusal of the available literature shows that the treatment of follicular mange in dogs, and in other species of animals, has been attempted along four different routes, as briefly indicated below.

#### I. Dressings.

The majority of the dressings so far recommended would appear to be of doubtful value and the results obtained by different workers from the use of one and the same dressing have frequently been so contradictory that from these records it is almost impossible to assess its true value as an acaricide. Hutyra and Marek [1926], in dealing with the subject of follicular mange collectively (i.e., without referring to the canine species in particular), list a large number of remedies that have been recommended from time to time by different workers. Some of these may be regarded as of quasi-mechanical character, being primarily designed to cause a loosening of the skin and detachment of the horny plugs in the hair follicles. Thus, one such remedy requires all fresh pustules to be squeezed out and later bathed in a 5 per cent. solution of liver of sulphur, or again, the pustules may be periodically fomented and rubbed with soap liniment, salicylated alcohol or salicylic oil or a 10 per cent. lysol vasoliniment. Hutyra and Marek also mention Peruvian balsam and Perugen (which latter is presumably a modification of Peruvian balsam), the former being mixed with alcohol before application and the latter applied in the form of an ointment in a strength of 10 to 20 per cent. So far as Peruvian balsam is concerned, it would appear to have been used with a considerable degree of success by a number of workers, and from these reports it would appear to be one of the most effective remedies so far introduced for the treatment of follicular mange of The authors also refer to two preparations of essential oils, one consisting of caraway oil (ol. carvi and alcohol āā 10 gm., ol. ricini 150 gm.) and the other of salicylated oil (1 part salicylic acid dissolved in 30 to 40 parts of warm oil), whilst the ointments mentioned by them include corrosive sublimate ointment (1: 100) carbolic ointment (1:10); a diluted ointment of cantharides (1:6 parts of lard) combined with a bath in liver of sulphur solution; and an ointment made of 20 gm. napthol, 0·25 gm. corrosive sublimate and 100 gm. lanoline, applied as a dressing after the affected parts have been bathed in liver of sulphur solution and then washed several times a day with lukewarm cresol emulsion. A somewhat unusual method of treatment referred to by Hutyra and Marek consists of pressing of pads of carbon bisulphide upon the affected parts after the latter have been prepared properly, this being followed by the application of a paste of formalin (formaldehyde 1 to 3, vaseline 50, zinc oxide and powdered starch āā 25). Of the comparatively simple remedies, Hutyra and Marek mention the application of creolin or ichthyol (with alcohol āā) and bathing in 1 per cent. solution of corrosive sublimate at 37°C. for 8 or 10 minutes (leaving the head free from the bathing fluid). The application of a liniment of soap and alcohol āā 100·0 gm. and 0·5 gm. potassium hydroxide is also reported to have yielded encouraging results. Finally, they quote Brandl and Gmeiner as having found liquor cresolis saponatus most effective, although Hutyra and Marek themselves failed to obtain confirmation of these findings.

Craig [in Wooldridge, 1923], although admitting the futility of measures hitherto recommended for combating follicular mange in dogs, refers to some succesful results having been obtained in the treatment of this condition from the use of autogenous vaccines and also by the hypodermic injection of Nuclein in the proportion of a minim per 1 lb. body-weight, once a week. Good results are also reported to have been obtained with various iodine preparations, such as Tr. Iodi applied twice a week. Craig quotes Hunting as having recommended a dressing made as follows:—

Creosote, 1 part.

Liquor Potassae, 2 parts.

Ol. Olivae, 14 parts.

The above dressing is applied every third or fourth day. Varying degrees of success are reported to have been obtained from the daily application, with a rag or brush, of a  $2\frac{1}{2}$  to 5 per cent. zinc chloride solution in water after the affected parts have been washed and dried. The use of a dressing of collodion and iodoform, with the object of asphyxiating the parasites, has also been recommended. A daily application of the following dressing has also been found beneficial:—

Ol. Carui,

Alcohol āā 1 part,

Ol. Ricini 15 parts,

combined with a weekly bath in a I per cent. solution of potassium sulphide,

Müller and Glass (l.c.) differentiate the method of treatment to be adopted for the pustular and the squamous forms of follicular mange in dogs. In the former, they recommend, in particular, Peruvian balsam or warm preparations of salicylic acid (1 part of salicylic acid to 40 parts of olive oil) or styrax (in oil solution). In the squamous form, they emphasize the desirability of endeavouring to reach the parasites by systematic rubbing with acid ointments or strong concentrated salicylic ointments (1 to 5), and also with soft soap and lye, and after the scabs and scales have been removed, the same treatment as prescribed in the pustular form may be applied. Besides, they mention a number of other remedies without, however, commenting on their actual efficacy for follicular mange. Their list of these remedies is reproduced below: - Cerate of cantharides and lard, 1 to 6; tincture of iodine and chloroform; oil of turpentine and chloroform; oleum petrae and alcohol; endermol, I per cent. solution; zymoidin in the form of ointment; silver nitrate, 5 per cent. solution; ichthargen, 10 per cent. solution alternated with iodine 10 per cent., and tar 25 per cent., each well rubbed in; formaline 2 per cent. solution; dermaform, oleoformaldehyde; creolin and alcohol, equal parts; liquor creositi saponis; creosote diluted with alcohol or sweet oil; oil of juniper; xeroform; carbonate of sulphur as a bath and then frequent applications with the powder and followed by an ointment, formaline 3.0, vaseline 50.0, oxide of zine and almond powder, each 24.0.

Aruch [1922] claims to have obtained "excellent" results in the treatment of demodectic mange in dogs with the following method:—The coat is brushed and thoroughly washed daily with tepid water, soap and creolin. In the case of dry coat, it is rubbed with cotton wool soaked in other and this is followed by a second rubbing with other containing 1—2 per cent. iodine, or 0.25 per cent. thymol, or 1 per cent. salicylic acid (but iodine is preferable). When the entire body is affected, it is advisable to divide it in 3 to 5 areas for consecutive treatment.

Barat [1926] recommends the following formula for the treatment of demodectic mange in canines:—

Carbolic acid, 10 grm.

Ether, 250 grm.

Chaulmoogra oil, 750 grm.

The animals are not washed during treatment. The application is made daily, without friction, for 5 or 6 days and then every 2 or 3 days, until hair begins to grow and treated cases are cleaned once a week with camphorated oil. A period of 3 to 6 weeks is usually necessary to obtain a cure.

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Alston [1933] reports having obtained marked success with the following method of treatment even in advanced cases of follicular mange in dogs:—The affected animal is washed in home-made lye soap and allowed to dry. It is then thoroughly massaged with a dressing made of

Lard, 1 part.

Sulphur, 1 part.

Phenol, added to make about 5 per cent. of the mass.

The massage is repeated once a week until 7 or 8 applications have been given. The method would seem to merit trials in this country, in view of the ease with which the remedy could be applied by the average dog-owner.

In a recent article, Crane [1933] gives the protocols of 13 dogs, in various stages of infection with follicular mange, treated with a compound containing rotenone (the parasiticidal constituent of *Derris* plants) as its active principle, the formula for this compound being as follows:—

Rotenone, 1 grm.

Alcohol, 50 c.c.

Acetone, 10 c.c.

Water, 40 c.c.

The rotenone is dissolved in acetone before adding the alcohol and water (as water does not dissolve rotenone and is used merely as a vehicle) and the resulting liquid is then stored in bottles which should be shaken before each application. The liquid is rubbed in vigorously over the affected parts, the application being made daily or three times a week (depending upon the nature and extent of infection). The results obtained in the treatment of the 13 dogs mentioned above would appear to have been surprisingly good, for "in eleven out of the thirteen cases in which it was used, rotenone caused an unusually rapid recovery from follicular mange. The remaining two cases recovered in the time usually necessary for the standard remedies. These cases included several which had not responded to the citrine ointment dressing which is the standard treatment in the Small Animal Clinic. It has the additional advantages of being entirely non-toxic and not greasy or dirty to apply ". An examination of the actual protocols of these dogs shows that the period taken to obtain complete recovery ranged from 12 days to about eight weeks and that in a few instances, one-half per cent., instead of the one per cent., solution of rotenone was used. Judged by these results, rotenone would appear to hold out the largest promise of success in the treatment of follicular mange of dogs and doubtless merits extended trials in this country.

Stephenson [1933] recommends the following procedure for the treatment of following mange in "small animals" (without, however, making any special mention of the cannine species):—

- "First, make a microscopic examination of skin scrapings and the contents of the pustules."
  - "Second, clean well with chloroform.
  - "Third, give mixed bacterin twice weekly.
  - "Fourth, give calcium sulphide internally.
- "Fifth, wash once or twice weekly with crudol preparation containing sulphides; in some cases alternate with chaulmoogra oil compound every five days.
- "Sixth, apply 20 per cent. silver nitrate solution to affected areas every 10 days until three treatments have been given.

Of the proprietary remedies so far introduced into the field for the treatment of follicular mange in dogs, mention may be made of the preparation "Odylen", which, although claimed by its manufacturers (Bayer Co., Germany) as being efficacious for all forms of mange, is particularly recommended by them for use in the treatment of follicular mange of dogs and other animals. The drug is rubbed in well over the affected parts and it is stated that 3 or 4 applications of the drug, at intervals of 5 to 6 days, are necessary to obtain a cure. It is further stated that the drug "has been found extremely useful in the treatment of follicular mange in dogs" when used in conjunction with injections (subcutaneous, intramuscular or intravenous) of "Arieyl" which, too, is a product of the same firm.

Some mention may be made of the dressings that have been used with varying degrees of success in the treatment of follicular mange in animals other than the dog, for some of these may be found suitable for the canine species as well.

Holmes [1920] recommends a combination of calcium sulphide and horse fat for the use in the treatment of follicular mange in the horse, the calcium sulphide being made as follows:—2½ lb. of sulphur and 1 lb. of quicklime are mixed together dry in a large bucket and hot water is gradually added, until the mixture is reduced to an even paste. The contents are then made up to 2 gallons with more water and boiled for about 3 hours until a reddish-brown solution is formed; this is then made up again to 2 gallons with water. One gallon calcium sulphide solution. 2 gallons water and 2 gallons horse fat are now well mixed together and applied over the affected parts, the compound being maintained at a temperature of 100°F, during the process of application.

Kappel [1925] reports having cured a case of demodectic mange in sheep by the repeated applications of equal parts of Sapoformol and water, although liniment of sulphur, creoline and oil (1:1:5) had no effect in this case.

#### II. FUMIGATION.

The possibilities of fumigation as a method of combating follicular mange do not appear to have been explored to the same extent as it has been done in the case of other forms of mange, notably in the case of scabies of the horse, where the method has been declared as "reliable, quickly effective, even on horses that are so far scabby that attempts at treatment are useless" [Hutyra and Marek, 1926]. While it is true that gas treatment can hardly be regarded as holding out much promise of success in affecting the parasites, imbedded as they are in the deeper layers of the skin, nevertheless, Uebele [cited by Hutyra and Marek, 1926] reports having obtained "surprisingly good results" after the administration of ozone which was directed to the affected parts through a tube from an ozone apparatus, although the animal manifested great pain.

#### III. DRUG INJECTIONS.

Nicolas [cited by Hutyra and Marek, 1926] recommends the injection of a 2 to  $2\frac{1}{2}$  per cent. carbolic acid solution into the depths of the skin (not under the skin), both in the affected parts and in the neighbourhood, the efficacy of this method of treatment being endorsed by Hutyra and Marek themselves and also by Craig (l.c.). It is of interest that Hardenbergh and Schlotthauer [1925] claim to have effected cure in a goat attacked with the pustular form of follicular mange by opening the nodules, emptying and destroying the contents and injecting into them a few drops of 10 per cent. carbolic acid and painting the immediate area with iodine. "The treatment was thoroughly carried out. About three weeks later only the remnants of two or three nodules could be found. Healing had taken place so that the previous site of the nodules could not be found".

#### IV. RADIANT THERAPY.

A form of treatment against follicular mange which has now come to be regarded with much favour is the so-called "Radiant Therapy". Motas would appear to have been one of the earliest to use Roentgen rays with success in the treatment of this condition, but Roussel found the method ineffective [Hutyra and Marek, 1926]. However, the most convincing proof of the efficacy of this method of treatment is afforded by the results of an intensive series of experiments carried

out by Marotel and Pierron [1926]. According to these authors, the best results in ultra-violet therapy are obtained with a mercury lamp ("model 2, type c") of 1,200 candle power, the animal concerned being "irradiated" for 50-60 minutes at a distance of 20 cm. As pointed by them, the ultra-violet phototherapy has a threefold advantage: (1) Like all baths in general (liquid or gaseous) the luminous bath most certainly touches all the affected parts, contrary to what would be possible with any form of manual treatment (e.g., friction, puncture, scarification, etc.); (2) ease of application; (3) absolute curative value, when the directions for treatment are rigorously followed (the authors treated twelve animals by this method and cure was obtained in every case). On the other hand, the authors mention three outstanding disadvantages of the method: (1) Risk of the skin being burnt when the patient is "irradiated" at a distance of less than 20 cm. or when exposed to radiation for more than 60 minutes or when the animal concerned happens to be a sensitive one (with a thin or insufficiently pigmented skin); (2) the head, because of the eyes, does not admit of being "irradiated", and one is therefore obliged to treat it apart by means of other remedies; (3) compared with other methods, radiant therapy is somewhat expensive.

In two recent articles, Hardenbergh and Sheard [1927, 1928] have published some observations upon the effects of ultra-violet and infra-red radiation on Demodex folliculorum in the dog. The observations, however, were mostly made upon the parasites in vitro and only a very small number of cases in vivo, their main conclusions in regard to the actual efficacy of the treatment being as follows: "The daily radiation of an animal affected with demodectic mange for from 15 to 45 minutes with an air-cooled quartz-mercury are lamp, operated at 90 volts at a distance of 50 cm., apparently maintains the general physical tone of the whole animal at a higher level and thereby aids in combating the untoward conditions set up in the host by the invading parasites". The authors regard it as possible that Demodex folliculorum is more susceptible to heat (infra-red) than to other forms of radiant therapy and that the benefits of this method of treatment are not due to any specific lethal effects on the parasites, imbedded as they are in the deeper layers of the skin, but to its stimulating action which causes a general improvement in the condition of the animal and thereby enables it to overcome the effects of the invading parasites. On the other hand, Marotel and Pierron (t.c.) describe the effects of ultra-violet phototherapy as follows: "After the first radiations, the skin tumefies and sweats ("suinte") at the level of the pustules which then become powdered ("saupoudres") over with tannin and this latter gets dissolved in the exudate, thus forming an acaricidal and astringent solution. At the end of the fifth day, the pimples disappear and the sweating commences to dry up and desquamate, and at the end of a fortnight, it attains a condition of simple dermatitis which

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follows its normal course. It is noteworthy that, according to Patton [1926], the areas to be "irradiated" should be "free from scurf, excretions, dirt, grease and so forth", and it is therefore possible that a lack of proper attention to this detail may have been responsible in the past for a proportion of the failures to obtain satisfactory results with radiant therapy.

Quite recently, Bannerjee [1933] in India has claimed to have obtained "most encouraging results" in the treatment of follicular mange in dogs by exposure to ultra-violet rays. The apparatus used by this worker was a mercury quartz lamp ("Hanovia"), the animal concerned being "irradiated" daily for five or more minutes on each side of the body at a distance of about 3 feet. The protocol of only one dog, however, is given and in this case the treatment comprised three courses, the first consisting of eighteen exposures and the second and third each of seven exposures, but skin scrapings continued to reveal the parasites until the commencement of the third course of treatment. Concurrently with the ray treatment, the animal was periodically subjected to "Pot. sulphate baths" combined with a skin dressing.

Removal of the affected parts, in cases of circumscribed infection, has been recommended by Roth (cited by Hutyra and Marek, l. c.), the method consisting in cutting the epidermal layer until drops of blood appear, the animal being anaesthetized.

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## A STUDY OF THE DATA OF MILK YIELDS OF VARIOUS TYPES OF CATTLE OBTAINED FROM THE RECORDS OF THE GOVERNMENT MILITARY DAIRY FARMS.

PART II. PERSISTENCY OF LACTATION AND ITS RELATION TO AGE AND LEVEL OF PRODUCTION.

BY

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(With six text-figures.)

In part I\* of this paper the milk records of herds of Indian and Cross-bred cows and buffaloes were analysed and it was shown that the milk yield may be expressed as a function of the two characters, namely, the initial rate of yield and the rate at which it declines. This rate of declines was used as a measure of persistency, (by which term is meant the degree to which the level of yield is maintained), the animal that declines most being the least persistent and vice versa. It is commonly known that the rate of decline varies with breed and with individuals in the same breed; it is affected by age, level of production, pregnancy, state of nutrition and other environmental factors; it is also affected by heredity. The rate of decline is thus subject to the same variations as milk yield or fat yield, and has to be studied as a separate character. In order to do this it is necessary to evolve a value of the rate of decline for each lactation; then alone can one find out the range within which this value varies in the several breeds, what its standard deviation is and how far it is influenced by the various factors enumerated above. In the following pages a preliminary attempt is made to measure the effect of age and level of production on the rate of decline, the study being based on a total of 812 lactations for each of which a value of the rate of decline was found out. The influence of pregnancy will be considered in a later section.

<sup>\*</sup> Part 1 of the paper was published in the Ind. Jour. Vely. Sci. and Anim. Husb. Vol. IV, Pt. 1, pp. 36-62.

Data of monthly yields had already been collected for the study reported in Part I. Some more were collected, making up a total of 344 lactations for the cross-bred, 244 for the buffaloes, 113 for the pedigree Sahiwal and 111 for the ordinary Sahiwals. The equation made use of to describe the lactation curve is of the same form as used in Part I, but the method of determining the constants (rate of yield and rate of decline) is slightly different, as the method employed there, though more accurate, is too laborious to be applied when hundreds of lactations are involved. The method is known as the method of averages, and an example of working is given in the Appendix.

The values of rates of decline are classified and given in Table I for the four breeds, and those for the cross-bred are represented graphically in Fig. 1. The

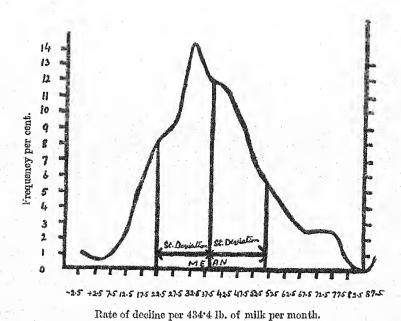


Fig. 1. Frequency distribution of the values of rate of decline. (Cross-bred cows.)

Table I.

Distribution of persistency values.

Persistency express as rate of decline 434·3 lb, milk per u	ssed per lonth		Frequ	ency	
Class mid-poin	t	Cross-bred	Pedigree Sahiwat	Ordinary Sahiwal	Buffaloes
-2.5	1	Angered Congress of State of Congress of C		- '	
2·5 7·5		4 2 3	3 3 2	1 2	 
12·5 17·5 22·5		8 19 28	4 7 18	 9 5	4 2 16
27·5 32·5 37·5	: :	31 49 41	8 13 14	11 4 8	23 23 46
42·5 47·5 52·5	: :	40 33 23	8 8 2	7 9 8	27 30 16
57·5 62·5 67·5		18 14 9	7 6 1	10 8 8	14 10 7
72·5 77·5 82·5		9 9 2	2 1	3 5 4	5 6 1
87.5 92-5 97.5	р	2	2 1 1	3	2
102·5 107·5 112·5			1 1	1	$\frac{\cdot \cdot}{\cdot \cdot_2}$
117·5 122·5 127·5			••	1 1 1	•••
Tot	tal .	344	113	111	244
Mean .		39.986	37*456	50.92	42.27

Note.—To save arithmetical labour the rate of decline is expressed per 4343 lb. milk. The percentage decline is obtained by dividing these by 4:343. These figures will be found in Table II.

TABLE II.

Means, standard deviations and co-efficients of variations of persistency, initial rate of yield and age in the various breeds.

Breed			declines month lb. milk		at ma	of yield per eximum (i.	e. at one	Mean age
	No. of records averaged	Mean	Standard devia- tion	Co-effi- cients of variations	Mean	Standard devia- tion	Co-effi- cients of variations	tions.)
Cross-bred cows . Pedigree Sahiwal . Ordinary Sahiwal . Buffaloes .	344 113 111 244	9·210 8·624 11·725 9·733	3·960 5·07 5·77 4·119	43:0 58:84 49:25 42:32	974·70 740·264 504·955 621·31	357·1 232·26 148·1 165·9	36.63 31.4 29.3 26.7	5·02 5·39 5·03 4·59

means and standard deviations are entered in Table II. Figure 1 shows that over 60 per cent. of the observations are scattered round the mean within the range indicated by the standard deviation. As was already noticed in Part I the specially bred pedigree Sahiwal at Ferozepore is the most persistent and the ordinary Sahiwal cow is the least persistent. The cross-bred and the buffalo are more or less on the same level. From the figures of coefficients of variation it would appear that the pedigree Sahiwals are most variable.

Table II contains also the rates of yield per month at maximum (which is yield per month at one month after calving). The cross-bred cow is the highest and is above the Ferozepore herd by 4 to 5 lbs. a day at their maximum, though a good deal of this difference is made up in the course of a lactation on account of their higher persistency. The buffalo is seen to be a distinctly better type of dairy animal than the ordinary Indian cow. Her rate of yield and persistency are both higher than that of the ordinary Sahiwal.

#### RELATION OF PERSISTENCY TO LEVEL OF PRODUCTION.

The strength of relationship between two factors is measured by the coefficient of correlation between them; and this coefficient can be calculated by well known methods. The value of this coefficient would lie between the limits 0 and 1, 0 indicating absence of, and 1 indicating perfect relationship.

The level of production is indicated by the initial rate of yield. Tables III to VI show the correlation between the initial rate of yield and the rate of decline.

<sup>\*</sup> The slight differences noticed between these values and those recorded on page 47 of Part I may be accounted for by (1) the difference in the method of determination of the values and (2) the increase in population.

TABLE III.

Rate of decline | Correlation surface shouring the relation of Rate of decline to Rate of yield. (Data: Cross-bred cows.)

per month per | Cross-bred cows.)

	Total.			বাঁ চন		00	38	31	44	80 80	18	66	C5 61	61	344	-
- G1	:	134.51	-	;	:	1	::	::	:"	Ě	; ;	::	::	: ;	=	42.2
23		23.5		: :	:	:	: :	::	: :	::	::	; ;	: :	: :	:	:
22	1	25.2		1 :	:	:	::	. : :	::	: :	::	: :	::	: :	:	:.
156	1	21.5	-	::	:	:	::	::	::	: :	::	::	::	11	:	i
20		20.2		: :	٠.	1	::	1:	* : :	۲:	::	, <del>, , ,</del>	::	11.	67	0.09
10	; ]	19.5	-	: :	:	:	::	::	::	::	::	::	¬ :	:: :		2.44
18	1	18.5		1 :		:		~61	::	::	::	::	::	:"	49	46.75
17	:	17.5		1 :	,	:	- : :	:"	::	e4 p4	::	:"	::	: :	1 4	51.75
16	1	16.5		1	: :	:	::	. : :	٠:	; C4	:	: ::	69 :	; ;	9	0.09
15	=	15.5		::		:	: :	<b>-</b>	prt ert	::	٦:	::	::	::	4	41.8
2		14.5	-	::	. :	i	::		ကက	-181	60 64	۳:	; ; ;	::	1%	1.84
133	;  -	13.5	0/1	::	:	:	; <del>=</del> .	::	40	<b>∵</b> ;	rd rrl	m ea	HH	; :	192	8.05
13		12.6		::	*	:	۲ :	. 65	ဗက	<b>69</b> 69	; ;H	: "	<b>H</b> :	: <b>: :</b> .	82	44.0
=	ij	11.5		: ;	:	:		41	64 10	00.00		: "	ea :	:7	88	9.25
10	; ; 	10.2		i și	:	:	:"	63 60	3G 15	याया	40	87-	:"	*::	3	45.0
6	-	9.2		; ;	:		८४ स	유디	65 51		63 H	1	::	11	4	<u> </u>
00		10		::	- ;	Ċξ	411	10 A	C/1 =-1	တ္တ	soI		-:	: :	88	34.3 35.7 35.1
7	1	7.51		;~	-	φ2 2	60.00	r-00	100	co ==	just tork	60 ←1	1:	::	92	14.3
9	-	6.5		7 :	H	<del></del>		mo.	63 64	H 63	Ħ:	mm	43	11.1	92	17.
10	1	6.9		iH	:	67	<b>с</b> р гч	<b>™</b>	:-	11	m ;	H:	1:	11:	7	27.5 37.1
#	-	4.5	18	Ä;	v-1		CQ (m)	eo :	10 63	₩ 63	, 1 1	1.1	11	11	9	30.0
63		53 C3		4.1	•	:	. pg pg	111	3 E		11	: 1	1.1	111	-4	20.0 30.6
64		10		<b>e</b> 1	- 1		:	- 1 .:	; i	; ;	. : }		11.	ij	က	6.6
	¥• <sub>1</sub>	Class mid- point.		61 64 62 64	7.8	12.5	17.6	32.5	20.00 20.00	47.5	62.5	87.5	77-5	92.2	Total .	Mean of columns .
		No.		0q	60	*	n to	K-00	00	HE	113	15.00	17	13		Mean o

TABLE IV. Correlation surface showing the relation of rate of decline to rate of yield . (Datapedigree Sahiwal cows.)

	Rate of d per month 434·3 lb of	ı per			Yield 1	per da <b>y</b>	in lb. a Class 1	t one m nid-poi	onth af nts	ter calv	ing	
No.	Class mid	.noint	1	2	3 .	4	5	6	7	8	9	Total
	(3110)333 314412	-Twatte	2.5	7.5	12.5	17.5	22.5	27.5	32.5	37.5	42.5	-
1 2	-2·5 2·5		1	1			1			1		3
3 4	7·5 12·5	17	••		1	1	3	1	•••	• •	::	2 4
5 6	17.5 22.5	:	••	1 1-		3	2 3	3 8	1 3		••	7 18
7 8	27·5 32·5	· :	••	••	2	1 1	1	2 6	2 2	2		8 13
10	37.5 42.5	:	•• ••••	¿.	1	2	2 1	5 3	4	2 2		14 8
11 12	47·5 52·5		- · ·		1		2	3	• •	1		8 2
13 14	57·5 62·5		*	::	*	••1	1	••0	3 2	2 1	1 2	7 6
15 16	67.5 72.5	•	••		••			2	••	• •	••	1 2
17 18	77.5 82.5		••	• •	• •	• •	• •	::	••		••	
19 20	87·5 92·5	•	*.	1		••	• ••	1	••		•••	2
21 22	97·5 102·5	•	•••	••		· · ·			. 1	••		1 1
23	107.5			••		• •		1	• • •	••		1
	Total		1	4	6	12	18	26	19	13	4	113
Mear	n of columns	-, , -	-2.5	32.5	26.7	37.5	43.6	37.6	39.0	47.1	53.8	

Mean rate of yield " decline 26°438 lb. per day. 37°4555 lb. per month per 434°3 lb. milk.

Standard deviation of yield decline 8:295 lb. 22:04 lb.

Co-efficient of correlation

2598.

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TABLE V.

Correlation surface showing the relation of Rate of decline to Rate of yield.

(Sahiwal cows. Ordinary).

	Rate of decline per month per 434:3 lb. of milk		Zield pe	er montl calvi	h in 100 ing. Clas	lb uniss mid-1	ts at one	e mont	h after	
No.	Class mid-point	1	2	3	4	5	6	7	8	Total
I e		2.5	3.2	4.2	5.2	6.5	7.5	8.5	9.5	_
1 2 3 4	12.5	1 3	2		3				*	1 2
5 6 7 8	37.5	2	2 2	2	5 1 3	1		,	•••	9 5 11 4
9 10 11 12	42·5 47·5 52·5 57·5		3 1 1		1 1 1 3	2 1 2	2 1 1			8 7 9 8
13 14 15 16	62·5 67·5 72·5 77-5	•••	2 1 	2 2 1 3	1 4 1 1	1 1 1	1	1::		10 8 8 3
17 18 10 20	82.5 87.5 92.5 97.5		••	1 2 	••	2	1			5 4 3
21 22 23 24	102·5 107·5 112·5 117·5		••	"1 .:		1	•	•••		1 1
25 26	122·5 127·5 : :	:	• •		1	1		••	::	1 1 1
	Total .  Mean of each column	9 23·15	17 38·7	32 54·85	26 47·5	15 68·9	9	2 80·0	1 117·5	111

# PERSISTENCY AND ITS RELATION TO AGE AND LEVEL OF PRODUCTION

## TABLE VI.

# Correlation surface showing the relation of rate of decline to rate of yield. (Duta:—Buffaloes).

	Rate of decline per 434'3 lb, milk.		Rate	of yield	per mo	outh in Clas	100 lb. s mid-1	units	at one	month a	after es	lving.		
No.	Class	1	2	3	4	5	6	7	8	9	10	11	12	Total
0	Mid-point .	1.2	2.2	3.2	4.2	5'5	6.2	7.5	8.5	9.2	10.2	11.5	12.5	- 7.
1	-2.5		1	1	1	3,							*	3
2	+2.5.													
3	7.5			1	2	2	1						***	6
4	12.5				2	1	1							4
5 -	17.5			1		1							<b></b> y	2
в	22.2			3		10	1	1	1				••.	16
7	27.5	1		1	7	3	6	4	1					23
8	32.5			1	6	8	6		- 1	1	119			23
9	37'5		2	. 2	, 9	12	. 8	11	. 1	1				46
- 10	42.2	***		3	3	8	12	3	2	1				27
11	47.5			,	1	6	7	9	2	- 5	•••			30
12	52.5		,		2	1	6	3	3	·		÷.,	1	16
. 13	67.5				2	2	3	- 3	3	1				14
14	62.5			1	1	1	2	3		2	•••			10
15	07.5		··· ,			2	3		1			- 1		7
. 16	72.5				. 2	,	1	2		·	•••			5
17	77.5				1	2	1	1	1					6
-18	82.5	, ···					·	1						1
19	87.5				1		. , 1	1						2
30	92.5	·					,	•••						447
21	97.5													***
22	102.2				***			•••	1					1
23	107.5										.,,		.,,	
24	112.2		,···	· )	1					. 1				2
- 7	Total .	1	3	14	10	54	59	42	17	12	•••	1	1	244
lean of	each column	27.5	24.2	29.7	38.0	37:4	44.0	47.8	51.6	53.8		67:5	52*5	***

Mean rate of yield per month

. 621'31 lb.

. 42.27 lb. per 434.3 lb. milk.

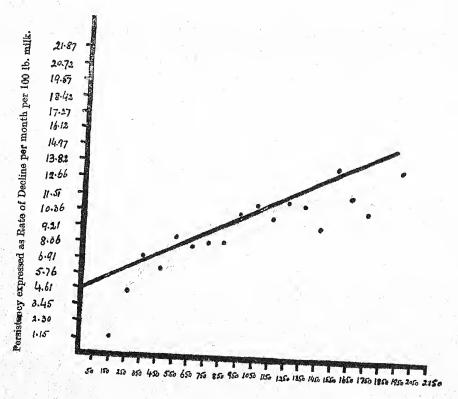
Standard deviation of yield . 165.9 1b.

decline

Co-efficient of correlation

3490

For convenience of reference the coefficient of correlation is entered below each table, as also the means and standard deviations of the two variables. The means of columns corresponding to each yield group are entered at the foot of the column. These means for Table III are plotted in Figure 2. The means are a bit irregular,



Rate of yield at one month after calving, in. lb.

Fig. 2. Relation of rate of decline to rate of yield at maximum. (Cross-bred cows.)

but they show a definite tendency to rise as the level of production rises. The best value for the variation in the rate of decline with variation in the level of production is calculated from the data and the thick line shows the calculated values. It will be noticed that the line cuts through the data very well especially between the 400 and 1,500 lb. groups (i. e. between about 15 to 50 lb. per day) which represent the most usual range of values of yield. Beyond these points the observations are scanty and the means are not reliable. The values of the rate of decline for the different breeds with increase in the level of production are calculated for all breeds and are entered in Table VII. The increase in the rate of decline

Table VII.

Showing the rate of decline per 100 lbs. milk at different levels of production.

Lo	vel of pre	duction	*	Rate of decline pe	er month per 100 lbs	. milk
. 3	yield per at maxi lbs.	month	Cross-b		Ordinary Sahiwa I	Buffaloes
150			. 5-2	261 5.271	5.977	5.643
250			. 5.7	795 5.839	7.595	6.512
350		,	6.3	6.407	9.213	7.381
450			. 6.8	6-975	10.831	8.250
550			7.3	97 7.543	12.449	9.119
650			. 7.9	8-111	14.067	9.988
750			. 8.4	8-679	15.685	10.857
850			8.9	999 9.247	17:303	11.726
950		*	9.5	9.815	18.921	12.595
1050		•	. 10.0	67 10.383	20.539	13.464
1150		* *	. 10-6	10.951	22.157	14.333
1250		- 1	. 11.1	35 11.519	23.775	15.202
1350			11.6	569 12.087		
1450			12.2	203 12-655		
1550	. ,		. 12.7	737 13-223	-	
1650		4.	. 13.2	271 13.791		••
1750			. 13.8	305 14.359	1000	
1850		• • •	. 14.3	339 14-927	*	
1950			. 14.8	373 15· <b>49</b> 5		

per 100 lb. for each 100 lb. rise in the level of production is

·534 in the case of the cross-bred.

568 ,, pedigree Sahiwal.
1.618 ,, ordinary Sahiwal.
869 ,, buffaloes.

This shows that the higher the level of production the less the variation in the rate of decline per unit of production. It is interesting to notice that the figures

are more cr less the same for the pedigree Sahiwal and the cross-bred, and it is not unreasonable to prophesy that the pedigree Sahiwal would lose her reputation for higher persistency when her level of production equals that of the cross-bred. The cross-bred also would have shown the same persistency as the pedigree Sahiwal if her production level had not been higher, as will be seen from the value of the rate of decline against 750 lb. which is the average for the pedigree animal. And indeed this has been found to be the case in a herd of 80 cross-bred cows of the Northern Circle which show an average initial rate of yield of 740 lb. per month and a rate of decline of 8.74 per 100 lb.

Table VII is valuable when persistency is made use of as the basis of testing if feeding and management are adequate or not. It shows what percentage decline may be normally expected at different levels of production. In making use of the table it should be borne in mind that the levels of yield shown against the rates of decline are productions at peak of lactation and not at any stage of it. Thus if a cross-bred animal is found to yield 750 lb. in the 4th month of lactation the drop in yield to be expected in the 5th month is not 8:465 of 750, but a higher percentage appropriate to the yield at peak. The yield at peak could well have been 1,000 lb. at which level the decline is about 10 per cent. So in all cases it is essential, when estimating the probable fall, to look up and see what the animal yielded at peak and find out the rate of decline corresponding to this level.

Table VIII shows the co-efficients of correlation between the three pairs of characters: persistency and rate of yield, persistency and age, and age and rate of yield. Persistency and rate of yield has been considered above. The relationship

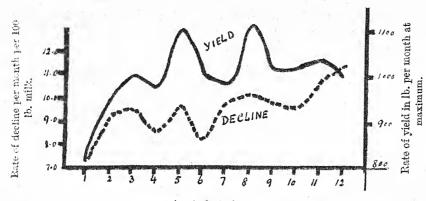
Table VIII.

Co-efficient of correlation between pairs of variables:—Rate of decline, Rate of yield,
and Age.

	Co-el	ficient of correlation between	'een
Breed	Rate of decline and rate of yield	Rate of decline and age	Age and rate of yield
Cross-bred cows Pedigree Sahiwal Ordinary Sahiwal Buffaloes	0·4817 0·2598 0·4153 0·3499	0·2352 0·3595 0·2204 0·1500	0·1674 0·0639 0·2243 0·1516

between age and persistency is weaker throughout except in the case of the pedigree Sahiwal, and that between age and rate of yield is still weaker. The correlation tables are given in the Appendix. The means of the values of persistency for each age group are shown by the dotted lines in Fig. III, a to d.

Broken lines show rate of decline, continuous line, yield.



Age in lactation.

Fig. III (a). Relation between age and rate of decline, and age and level of production in crossbred cows.

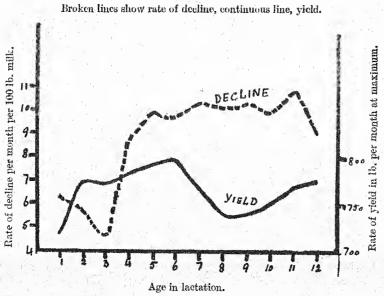


Fig. III (b). Relation between age and rate of decline, and age and level of production in pedigree Sahiwal cows.

Broken lines show rate of decline, continuous line shows yield.

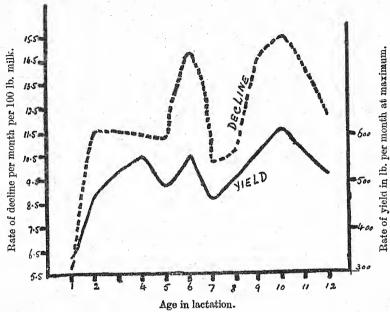


Fig. III (c). Relation between age and rate of decline, and age and level of production in ordinary Sahiwal cows.

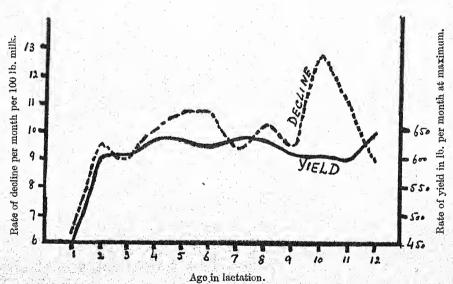


Fig. III (d). Relation between age and rate of decline, and age and level of production in buffaloes. Broken lines show rate of decline, and continuous line, yield.

They are found to be very irregular and the relationship is by no means linear. The outstanding feature of these graphs is the marked rise, in all breeds except the pedigree Sahiwal, from the first to the second lactation showing that heifers are definitely more persistent than older cows. From the second to the third lactation the rise is less marked, and thereafter the curves, though very irregular, generally tend to be flat. The continuous line shows the mean initial rates of yield in the various age groups, and it is interesting to observe that the irregularities in the two are in the same direction, a fall in the rate of yield being followed by a fall in the rate of decline, and vice versa. This suggests the explanation that persistency is not influenced by age, but only by the level of production. The marked rise from the first to the second lactation in the rate of decline is probably due to the initial undeveloped condition of the udder, on account of which the heifer starts with a low yield, but, as her udder is gradually developing, her milking capacity also increases gradually, and this increase masks a portion of the decline in milk yield. Her persistency, therefore, appears to be high.

Table IX shows the partial correlations between the variables, that is the net correlation between two of the variables when the third is eliminated. When the

TABLE IX.

Co-efficients of partial correlation between any two of the variables: rate of decline, rate of yield, and age, when the third is eliminated.

	Co-	Co-efficient of partial correlation.										
Breed	Rate of decline and rate of yield	Rate of decline and age	Age and rate of yield									
Cross-bred cows	•4615	•1789	•0635									
Pedigree Sahiwal cows	2543	· <b>3</b> 558	•0327									
Ordinary Sahiwal cows	-3850	1435	-1496									
Buffaloes	3347	•1050	•1070									

effect of the level of production is eliminated the correlation between age and persistency becomes definitely insignificant. The pedigree Sahiwal is an exception to this generalisation—the rate of decline actually decreases from the first to the third lactation though the rate of yield is increasing. But no satisfactory explanation could be offered for this departure. The number of observations in each group are no doubt very small but so is the case in the ordinary Sahiwal where the rate of yield and the rate of decline are found to follow the same trend.

#### SUMMARY AND CONCLUSION.

Values of rate of decline are found for 812 lactations of the cross-bred cow, the pedigree Sahiwal, the ordinary Sahiwal and the buffalo. The values are found to lie within a range of 1 to 20 per cent. decline per month. The average rate of decline varies from 8.6 per cent. in the case of the pedigree Sahiwal to 11.7 per cent. in the case of the ordinary Sahiwal. The cross-bred and the buffalo show 9.2 per cent. and 9.7 per cent. respectively.

The rate of decline is found to increase with rise in the level of production. Tables are given showing the percentage monthly decline to be expected at various levels of production in the different breeds.

The rate of decline is found to increase definitely from the first to the second lactation. From the second to the third the increase is less marked, and thereafter the curves tend to be flat. Beyond this no definite age—persistency relation is indicated. The mean persistencies for each age group, though irregular, follow the mean rates of yield for the same group very closely, indicating that persistency is influenced more by level of production than by age.

#### REFERENCES.

Associates of Rogers (1928)—Fundamentals of Dairy Science.

John W. Gowen (1924)

(also those cited in Part I).

#### APPENDIX I.

# DETERMINATION OF THE MAXIMUM RATE OF YIELD AND THE RATE OF DECLINE BY THE METHOD OF AVERAGES.

The example below illustrates the method of working when the number of observations is 10. When the method of averages is employed it would be more convenient and simple if an even number of observations is chosen. In all cases the observations are divided into two equal halves as shown. These are added up and the difference of the totals is found (total of second half is always subtracted from total of first half). In the case of 10 observations this difference has to be divided by  $25 \times 4343$  to obtain the rate of decline per lb. per month.

With 8 observations the divisor is  $16 \times 4343$  and with six the divisor is  $9 \times 4343$ . These are the cases ordinarily met with. But generally with n observations the divisor would be  $n^2/4 \times 4343$ , where n is an even integer.

To find maximum:—When the number of observations is 10 (see example below) 10/25 of the difference has to be added to total of first half. 1/5 of the sum gives the log of maximum.

With 8 and 6 observations 6/16 and 3/9 respectively of the difference should be added to total of first column and the sum divided by 4 and 3 respectively to obtain log of maximum.

Example.

Determination of rate of decline by the method of averages

Time after reaching maximum	Yield in lbs. per month	Logarithm of yields	First half	Second half
0.	1,272	3.1045	3.1042	2.9600
1.	1,135	3.0550	3.0550	2.9320
2.	933	2-9699	2.9699	2.8739
3.	911	2.9595	2 <b>·9</b> 595	2.8470
4.	922	2:9647	2.9647	2.7868
5.	912	2.9600	15:0536	14:3997 (Totals.
1),	855	2.9320	- *	
7,	748	2.8739	, , , , , , , , , , , , , , , , , , ,	
8-	703	2.8470		
9.	612	2.7868		8 1.,
	V	Difference	(First half—Second	half)= 6539
	-	25	divided by 25	- 02616
		13	,, ,, and	
		-	by ·4343	0602-The
*		rate of decli	ne per lb. per month.	•

 Muximum:
 Difference
 = 6539

 10/25 of difference
 = 2616

 Adding to total of first half:
 = 15.0536
 + 2616

 = 15.3152

 Divided by 5= 3.0630
 This is the logarithm of the maximum.

 The maximum is:
 1156.

## APPENDIX II.

Tables of Correlations :-

- (a) Between Age and Persistency.
- (b) Between Age and Level of Production

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TABLE I.

Correlation surface showing the relation of rate of decline to age. (Data:—Cross-bred cows.)

No.	Rate of decline per 434'3 1b. milk	100				A	ge in la	ctations						Hadrod Hill Testing Typ		
	Class Mid- point	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
								1	ĺ		·	1	·		-	-
1	-2.5		1	1	1	<b> </b>		<b> </b>	0	<b> </b>			1			
2	+2.5	1		٠.		1	<b> </b>	<b> </b>			::	•••		1	+	4
. 8	7.5	1			1			1						"		2
4	12.5	2	2	1		1	<b> </b>		1		1	::	"		•••	3
5	17.5	8	2	1	3		2	1			1	::	" 1	"	"	8
6	22.5	11	4		3	1	1	3		2	3		1	''	1	19
7	27.5	8	3	5	4	1	3		1	4	2	::	"	"		28
8	32.5	Ò	10	. 5	5	3	2	2	2	2	4	3	"	1	1	31
9	37.5	6	8	7	3	8	3	1	1		3	1	::		-	49
10	42.5	5	5	5	5	3	3	. 1	2	4	1	4	1	1		41
11	47.5	5	3	4	3	5		2		1	4	3	1		1	40 33
12	52.5		3	3	1	5	1	2		3	2	2	1		"	23
13	57.5		5	2	. 3	3	1				1	1	2	"	"	18
14	62-5	1		2	3	1	.1	. 1	2		2			1		14
15.	67.5	- 1	2	1		••=				1		1	3			9
18	72.5	2	. 1		1	1		1			1		1	1	10	9
17	77.5	•••	•••	2			••	1		2	2	1		1		0
18	82.5	•	1			•		1.								2
19	87.5	•••		1.0	٠					·					1	
20	92-5		1					,	-1			٠				2
. 1							, i			·	÷			''		"
Tot	al .	60	51	39	36	33	17	17	10	10	27	16	11	7	1	344
	of each umn	31-4	39•95	41•4	37 <b>·</b> 5	42.4	36.3	43:1	44.2	43.0	42.3	47.2	49-8	54.7	32.5	

меанаде

5.07 lactations.

Standard deviation of age

3.58 lactations.

Mean rate of declir

39.99 lb. per month per 434'8 lb. milk. Standard deviation of decline .

17:00 1h

Coefficient of correlation

TABLE II.

Correlation surface showing the relation of rate of decline to age. (Data:-Pedigree Sahiwal.)

olin	of de- e per 3 lb.						Age in l	actation	ns						- ;	
No.	Class Mid- point	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
1	-2.5	3									.		·			3
2	+2.5	1	1				1	••	**	••	- ;	٠.				2
3	7.5			ı.					. 1	••	••	••				4
4	12.5		1	ı	1				••	••	1	••	••		••	7
5	17.5		1	3		••		1	1	1		••	2	• • •	••	18
6	22.5	3	4	2	3	1		•••		1	••	2			• 7	8
7	27.5		3	1	2	••	1	1	••	••	••	••	••	1	••	13
8	32.2	2	2			4	2		2	••	••	••	**	••	1	
9	37.5	1		1	1	2	2	2	3	1	••	1	••	••	••	14
10	42.5	4			2			2	••	••	••	•••	••		**	8
11	47.5	1			1	1	••	••	••	2	១	••	•••	1	•••	8
12	52.5	1			٠.	••				•:	1	••	••	••	*	2
13	57-5	٠.,	1	٠.	1		1	2	1	••	1	••	••	••		7
14	62.5				2	2	1	1-	••		••	••		••	••	6
15	67.5		٠.			••		. 1	••	••		••	••	•••	••	1
16	72.5					1.			••	••	••	• •	1	•••	•••	2
17	77.5						*		••			••		••	••	••
18	82.5				1	•••			••	٠٠.		••		•••		1
19	87.5						1		1			••				2
20	92.5							••	1	••	•••	• •,,			••	1
21	97.5								*	1		•••	•••			, 1
22	102-5				••					••		••			1	1
23	107.5	٠		٠.	••			••0	••	• • •	••	. 1	•••		••	1
To	tal .	16	13	9	14	11	9	10	10	6	5	4	3	1	2	113
C	n of each	27:2	25.2	20.3	37.8	43.0	42.0	45.0	44.0	45.0	43.0	47.0	39.1	47.5	67.5	-

 Standard deviation

Coefficient of correlation

. 318

Standard deviation

. 22

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TABLE III.

Correlation surface showing the relation of rate of decline to age. (Data: - Sahiwal cows.)

Mai					power state and a	Spatial management	designation of the second			,							
N	Rate deel per 4 lb. m	ine 34°3							Age in	lactat	ions						
	Clas Mic poir	1-	1	2	3	4	5	6	7	8	9	io	1	1   1:	3 1	3 1	1 Total
	1 :	.5	1					-	-			1-	-	- -	- -		-
	2 7	.5	1				,	- 1		"	•••		"	•   •••	٠   ۰۰	•   "	. 1
	3 12	5							"	"	•••	"	"	.	'   "	·   ···	2
	4 17	-5	1	1	2	3				"	•		"			٠	
·	5 22	.5	2	1			,   '	1		"		***	"			"	9
. 6	3 27	.5	1	2		3	1	3 .	1	",		•••	"	"	"		5
- 7	32	5	1		2		1 .	- 1	- 1			***	"	""			- 11
. 8	37	5 .		1	2	1		- 1	1	3		•••	"	, · · ·	.		4
9	42	5 .	.	-2 .		1	1	1	1	1		• • • •			"	· '''	8
10	47	5		-1	3 ,		1	1			1	 1	"	1	*`		7
11	52	5 .		1 .	. ]	- 1	2				1	2	"	""	"	***	9
12	67	5	.	2	3	.	1	- 1	2	1	1	_	"	",		- "	8
13	62-1		.   .		2	2		1			]	•••		1		"	10
14	67.1	5	1 .		.	1	2	1		1	1	***		1	-	12	8
15	72.5	· /	.   :		:	1	ı		1.			1					8
16	77-5			1	1		-	-1	J.,		2	- 1	***			*	3
17	82.5		· ·	.	2	1	١,,,	",	1		.	"	•••		"	"	5_
18	87.5		일함	1	.	1	-1	1	"				***				4
19	93.2	1				12				T	. 1	2		1 7		,	3
20	97.8				-		- 1					*	·			1	
21	102.5		1	1			l			1	ł			0	•••		1
22	107.5		1				1	4, 1	1	1			,	*			1
23	112 5					1		1	1		-				***	: ••• ×	1_
24	117.5									-	.		5,0			*	
25	122.5	1		-	ile s		·					1	.,,	***	al."		1.0
26	127-5			1	1		0-									a	1
-	otal .	8	14	-	16	16	10	. 8	7	-		5		3	***		111
ean column	of each	25.0	50.0	50'0	49.7	48'8	64.5	45.3	47.5	65	69			54.5	***		1
	n ago	Mark to the state of		1	1		أجرين	1		1	1.	1					

TABLE IV.

Correlation surface showing the relation of rate of decline to age. (Data: -Buffaloes.)

No	Rate of decline per 434'3 lb, milk		, ,	×	n suspensy de la company de la			Age	în laet	ations	7	-	*	-	*	
	Class Mid- point	1	22	3	4	Б	6	7	8	9	10	11	12	13	14	Total
- 1	-2.5	1		3	1		***	1		***						3
2	+2*5		1	<b> </b>						×		F				*
3	2.5	2	,	2	1											6
4	12%	l	3	1											*	4
5	17.5				1					1						2
6	22.0	2	2	3	5		1	1	1				1			16
7	27.5	3	3	4	5	1	3	2	1	1						23
- 8	32.5		- 5	5	- 3	2		3,	1	1	3		1			23
9	87.5	i	10	9	5	3	6	3	6	2	1				ļ	46
10	43.5		7	4	5	2	1	5	2				1			27
11	4715	2	5	6	4	7	3	+1		2						80
12	52.2		5	4	1			1	3	2	***			1 1-		16
13	57.0		4	2	2	1	3	i.					1	1		14
14	62.5		1	2	2	1	1	1	- 1	1	1				,	10
15	67.5			1		- 2	1	- ***	1			2				7
16	72.5		1		2		1	·	1							. 5
17	72.5		2	1	2			.,,	1	·		***				- 6
18	4 28				1									,,,		1
-19	87.5			1			I		147							2
20	92.5		2.00					***	20					,41	***	×
21	117.7				١		ē			S.,			.,,			
22	102.5						×	1	S		11. de				· .i.	1
23	107.5	, x = 1			*		.,,	*	.,,				<i>.</i>			· •,,
24	112-5			NA COMPANS SANS	1	***	(W.)	***	•••	***	1	*				2
Total		10	49	47	40	19	21	20	- 10	10	5	2	4	1	***	244
Mean colur	of each	27.5	41.4	39 1	43.8	46*2	46.6	41.0	41.7	41*5	22.2	67.5	38.3	52.5		

Mean age 4.5943

Mean rate of decline 42.2705 Standard deviation

2.601

Standard deviation

17.890

Coefficient of correlation 1500

# 144 THE INDIAN JOURNAL OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY [ IV, II. TABLE V.

Correlation surface showing the relation of rate of yield to age. (Data:—Cross-bred cows.)

No.	Rate of Yield in 100 lb. unit	,		*		-	Ag	e in l	nctation	15	-			*	-	
×	Class Mid- point	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
1	1.2													••	٠,	
2	2.5		••	1							1		1			3
3	3.2	2	••					1			1					4
4	4.5	4	4	1	4	2	1	1		1	1		<b></b>			19
, 5	5.5	5	2	2	2	1				2	•••					14
ø	6.2	8	4	5	1	2	1	1		2	2					28
7	7.5	14	8	5	5	3	2	13	1		3	3	1	••		48
8	8.5	7	8	3	6	2	1	2		2	5		1	1	1	39
9	9.2	7	6	5	2	4.	5	2	2	1	2	5	1		••,	42
10	10.5	6	6	4	4	3	- 1	3	4	3	1	3	3	1		42
11	11.5	2	3	4	4	4	. 8	2	1	2	2	2	2	2		83
12	12.5	. 8	2	2		4	1		1	2	1	2	٠	2		20
13	13.5	+ 1	5	2	8	1				1		٠	1	1		15
14	14.5	1	1	2	1	3	1			2	5	·:•	1	***		17
15	15.5	×	٠. ا	••	3	••				• • •	1	:		٠	••	4
16	16.2		• • •	- 1		1	1			1	1	1				6
17	17.5		1.	•		2	••	.,:			1					4
13	18-5	(	1	1	1.	••			1					••		4
19	10.5			••				1					••	- ' • • •		1
20	20.5	٠,,	••			1	••	1	•••	1.	,,					2
21	21.2	••,		ું ••			••		••	2.0						_
22	22*5		•	٠.	,		••		••	••		••		••	••	_
28	23.5	• •	•	••	×	_ · ·	••		••			••	•••	"		
24	24.5		••	1	••	••	••	-3	0 · ·			<b>A.</b> •	2.4	••		1
т	otal .	60	51	39	36	33	17	17	10	19	27	16	11	7 -	1	344
Mear per	yield month.	812	942	999	975	1096	1^03	985	1110	1018	1020	1038	1005	1150	850	

TABLE VI.

Correlation surface showing the relation of rate of yield to age. (Data:-Pedigree Sahiwal.)

No.	Raic of Yield per day		,		-		A	ge in la	tetation	S _	· · · · · · · · · · · · · · · · · · ·		11		- x	
-	Class Mid- point	1.	2	3	4	5	6	7	- 8	9	10	11	12	13	14	Total
1	2.5	1	**	••		•				••						1
2	7:5	1	-	••-	•	••			2	1			'			4
3	12.5	5	• •	••					1	•••	 a				-	6
4	17:5	2			2	••	1	3	χ.	••	1	1		1	1	12
5	225	3	4	1.	1	1	*	2	1	3	2		••		••	18
6	27.5	4	4	6	4	5	3	1	4	1	1	1	2	•	••	36
7	32-5		4	2	3	. 2	••		2	1	1	2	1		1	19
8	97-5		1	••	3	1.	4	4	••	,.	•	••	••	••		13
9	42.2		*		. 1	2	1	,.	•	••	••	•	••		••	4
	Total .	16	13	()	14	11	9	10	10	6	5	4	3	1.	2	113
Mea	n yield	25.0	27.7	27.6	28.0	25.3	28.5	27.5	26.5	26.2	26-9	27.5	27.8	25.5	27.0	
Mea	n yield or month	716	774	773	784	793	798	770	742	742	753	770	779	714	756	- 30 - 1

Standard deviation . . 3.369

Hean rate of yield per day at maximum . 26:438 lb.

Standard deviation . . 8.295 lb.

Coefficient of correlation

. '0639

# 146 THE INDIAN JOURNAL OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY [ IV, II. TABLE VII.

Correlation surface showing the relation of rate of yield to age.

(Sahiwal cows.)

. Allege	erespionenses	Philippin Property					(San	iwal .	cows.	)					
N	Rate of yield i 100 un lbs.	of n it	, , , , , , , , , , , , , , , , , , ,					n lacta	on the state of th		= = 1			finimal objects and	
-	Class M poin	id-	1	2	3	4	5	6	7	8	9	10	11	12 / 1	3 Total
			10		-					_				-	- Total
1	1.5	-	•   •		.		.	.   .	.   .	.   .	.	.   .	.   .	.   .	
2	2.5		3	2				0							
					1	1	1		1 .		•   •	•   •	•   •	•	9
3	8.5		3	2	3	2,	3	1	2	1	٠   .	.	.   .		17
4	4.2			5	1 :	2	5 ,	1	2	2	5			1	-82
5	5.2	-	-	2 8		3 4							0.0		
	-						1			3	1	3	2		26
6	6.2		5	2	2	2	3	2	1					5	15
7	7.5		1	3	2	1						-	-	. E1	
8	8•5		-		1								10.2		9
	0.0		-		1	) :	1				٧.				2
9	9.5					1.1				1		1			1
- 1	Total .	8		-	1 0				-						
	7-34		14	16	10	16	10	8	7	8	5		3	•	111
7	Mean rate of yield			***		- 1		1.4						7	
7 T	in lbs.	338	471	519	551	488	550	463	507	563	610		517		31.

Mean rate of yield per month at max. 504.955 lbs. Coefficient of correlation

Standard deviation Standard deviation

2.749 148.1 lbs.

Table VIII.

Correlation surface showing the relation of rate of yield to age. (Data-Buffaloes.)

No.	Rate of yield in 100 lb. unit						Ag	e in lac	tations				×		
	Class Mid-	ı	1 :	3	1	5	_6	7	8	9	10	11	12	13	Total
	p <sub>rops</sub> may of greet controlled to the color of			Tive:						. : x .					
1	1.9	1				-	••			- *	••	••	••		1
2	24	1	1	1		٥.	••			••		••	••		-3
8	3:5	2	3	21	3		2		1	1		••			14
4	4°b	3	8	7	3		5	5	4	3	1		1		40
5	5.8	1	10	10	14	5	2	4	2	1	3	1	1		54
ti	6.0	1	15	17	7	6	3	3 ,	3	2	••	1	1 .		59
7	7.5	••	8	7	6	7	7	4	2	1			· *	×	42
Ŗ	815	1	- 2	2	3		1	4	3	1	••	••			17
9	9.5		2	1.	4	*	1	*	1	1	1		1		1.2
10	10 5			••	••	•		••	·	•••					••
11	115		.,-			. 1	,•••								1
12	12:5 -	•••							•••				٠٠ ۽	1	1
	The second secon	1 16		-	-		- 2								
	Total .	10	40	47	40	19	21	20	16	10	5	2 -	4	1	244
Mean	yield per nonth lb	450	607	607	638	687	621	640	639	610	610	600	650	1.250	

### ON THE OCCURRENCE OF SCHISTOSOMA JAPONICUM KATSURADA IN INDIA.

 $\mathbf{B}\mathbf{Y}$ 

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(Received for publication on the 10th April 1934.)

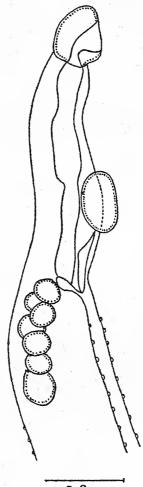
With one text-figure.)

Through the courtesy of Dr. P. A. Maplestone an opportunity was afforded to examine some schistosomes obtained from the intestine of a pig in Calcutta. Only about half a dozen male specimens in poor state of preservation were received and these were studied *in toto* preparations only. No female was represented in the collection.

The male worms measure 5.65—6.62\* in length and their maximum thickness varies from 0.183 to 0.258. The cuticle of the body is entirely covered with tubercles of moderate size bearing a varying number of minute spines on their summit. The number of the spines on these tubercles vary from 1 to 8, indicating that they are easily lost during manipulation. It was not unusual to find some of the tubercles entirely bereft of the spines. In addition to these tubercles the ventral surface of the body is covered with minute spines.

The subterminal oral sucker measures  $0.147-0.167\times0.137-0.153$ . The pedunculated ventral sucker is 0.16-0.167 in diameter and 0.053-0.06 in thickness. Both the suckers are covered with minute spines and the distance between them is 0.263-0.293. The funnel-shaped mouth leads into the esophagus measuring 0.31-0.32 in length. The intestinal execumite at a point somewhere between one-fourth and one-third of the body length from the posterior extremity. The common intestinal execum terminates at about 0.132 from the posterior end of the body.

The testes are seven in number and measure  $0.052-0.1\times0.052-0.094$ . They extend posteriorly for a short distance from the commencement of the gynæcophoric canal. When observed laterally under high magnification five of them, viz., the first, third, fifth, sixth and the seventh appear at one level and the remaining two, viz., the second and the fourth appear to be situated at a slightly different level. The vesicula seminalis appears to be an oblong sac, measuring  $0.047-0.05\times0.024-0.033$  and opens to the exterior by a short canal.



0.2 mm.

Lateral view of Schistosoma japonicum.

Although the schistosome group on account of its extreme economic importance has evoked in recent years considerable interest and has been an object of investigation at several places in this country the occurrence of an adult Schistosoma japonicum has not, up to the moment, been recorded from any host in India. It is, however, very significant to note in this connection that Sewell [1919] described cerearia resembling in almost all the details that of S. japonicum from the molluscs Indoplanorbis exustus Desh. and Limnua amygdalum Troschel in Calcutta. The differences between this cerearia and that of S. japonicum were, to quote Sewell "Very slightly smaller as regards body and a trifle longer in the tail, but owing to

the degree of contractibility possessed by the animal these differences are of a slight character as to be negligible." "The differentiation of the cephalic gland cells into coarsely-granular and finely-granular cells is a physiological rather than a morphological difference." Sewell further remarks that "the final test of the identity of this form with that of Schistosoma japonicum lies in the similarity or otherwise of the adults". The present form from the pig was found on careful examination to resemble in almost all the respects S. juponicum particularly in respect of the alimentary canal and the number of testes which in all cases were typically seven. The only points of difference were the smaller size of the worms and the tuberculate nature of their cuticle. To the question of the difference of dimensions much importance cannot be attached, dependent as it is on several factors such as the contraction, age of the worms, etc. In regard to the tuberculate nature of the cuticle my recent studies on this group of worm [Bhalerao, 1932], have shown that this feature has not much significance, variable as it is within the limits of the same species. Although some workers such as Montgomery [1906], Price [1929] and Brumpt [1931] have regarded this feature as of importance in differentiating one species of schistosome from the other, my previous studies on S. spindalis and Ornithobilharzia turkestanioum have indicated conclusively that this feature is not constant even within the range of the same species. The present example only forms an additional support to the conclusion that I had previously reached in this connection. In conclusion it may be remarked that both these differences merge into insignificance in so far as the specific identity of the parasite is concerned and for this reason it is considered that the specimens from the pig in Calcutta are S. japonicum. Considered from the view point of geographical distribution it is quite appropriate to expect the occurrence of this fluke in India as it occurs in the adjoining countries in the eastern zone of the Asiatic continent, viz., China, Japan, Formosa and Phillipines and Africa in the west.

In the end one can not fail to appreciate the fact that both the cerearia and the adult have been recorded from the same locality, viz., Calcutta. One can, moreover, correlate the smaller size of the body of the cerearia with the smaller size of the adult worms compared with the larger size of the cerearia and adult of a typical S. japonicum. It is more than the scope of the present communication to correlate the tuberculate nature of the cuticle with any structure in the larval organization.

One fact, however, seems to be apparent that although specifically the structure and the organisation of the adult and cercaria of Schistosoma japonicum are the same as those of the adult schistosome of pigs in India and their supposed cercaria from Indoplanorbis exustus and Limnua amygdalum, the differences of the size and the nature of the cuticle are sufficient to distinguish one variety of the species from the other, but the writer is not in favour of creating several varieties within the

limits of the same species. This unnecessarily adds to the worries of a systematist in such a complicated and a chaotic group as the parasitic worms. In all such cases it is a more desirable procedure to record different variations occurring under the same species rather than to multiply several varieties for various deviations noticed. The writer had adopted this procedure even on previous occasions in the cases of Ornithobilharzia turkestanicum and Schistosoma spindalis.

It is rather surprising in the end to notice that in spite of the occurrence of S. japonicum in pigs there exists up to the present moment no record of the presence of this fluke in human beings in this country. It is, however, likely that future investigation will reveal the presence of this parasite also in man like many other porcine parasites which are common to human beings.

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## SELECTED ARTICLES

### THE DIGESTION AND UTILISATION OF CRUDE FIBRE.

 $\mathbf{B}\mathbf{Y}$ 

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Crude fibre may be defined as the sum of all those organic components of the plant cell membrane and supporting structures which in chemical analysis of plant food-stuffs remain after removal of crude protein, crude fat and N-free extractives. The definition of crude fibre set up by Henneberg and Stohmann (44) is a convention, based on the method devised by them and known as the Weende method for estimation of crude fibre, which consists of the residue after treatment with 1.25 per cent. sulphuric acid, 1.25 per cent. potash, water, alcohol and ether. This includes, like the plant cell membrane itself, a mixture of chemical substances, celluloses, hemicelluloses (pentosans, hexosans) and encrusting substances (lignin, cutin, suberin, pectins). Investigation of the chemical, and even more of the physical and structural, properties of these substances is as yet incomplete (cf. 93, 96, 62, 67).

The digestibility of crude fibre both by animals and man is greatly affected by the wide differences, qualitative and quantitative, in make up of the cell membrane from these components. Digestibility of crude fibre is of great importance with regard to the utilisation of plant food-stuffs, for the energy supplied by crude fibre, which consists chiefly of carbohydrates, can be utilised only if the fibre is digested. Further, the contents of plant cells can be utilised only if cell membranes, which are easily permeable to digestive enzymes only in the more delicate structures, can be ruptured mechanically or chemically dissolved.

#### ENZYMIC DIGESTION.

In nature, solution of cellulose by animal digestive enzymes is of minor importance. Cellulases and hemicellulases capable of achieving this, have been found by Biedermann and Moritz (3, 4, 5) in the snail, Helix pomatia, and in the fresh water erab, Potamobius astacus. According to Dore, Boynton and Miller (19, 7, 91) the shipsworm, Teredo navalis, digests wood fibre by cellulase. Lewis and Jewell (74) found hemicellulases in 20 different species of insects and the existence of cellulases in protozoa must be assumed (V. Buddenbrock (12), p. 585).

Digestion of the cellulose of lichens lichenin (63) in *Helix* (64), *Potamobius* (72) and even in mice (141) and rabbits (136) has been found to be due to a digestive *lichenase*. Lichenase occurs in lichens and other plants; indeed, mobilisation of reserve celluloses is dependent on the occurrence of cellulases and hemicellulases. On the other hand, in animals, the cellulases which occur as enzymes in food, contrary to the original view of Brown and Morris (10), do not take part in the digestion of fibre (115), even if the animal body provides favourable conditions for the action of plant cytases (145).

In almost all cases where animals eat much plant food, the chemical digestion of fibre is due to the *enzymes of symbiotic micro-organisms*. Of these the most important are flagellate infusoria and bacteria. According to the observations of Cleveland (13, 14), the digestion of wood by termites and by wood eating fish (15, 135), is due to the production of an active cellulase by intestinal *flagellates*.

Symbiosis with cellulose splitting bacteria occurs widely in man and animals that subsist on plant food. In farm animals the bacterial digestion of fibre in the feed plays an important part with regard both to the availability of plant cell contents and to the utilisation of the energy of fibre.

The process of digestion of fibre is best understood in ruminants. It was first observed by Sprengel (125), described by Haubner and Sussdorf (40), and subjected to exact chemical study by Henneberg and Stohmann (44), who found in cattle a digestibility of fibre in meadow hay of 57-65 per cent., in oat straw of 55 per cent. Soon after Wildt (144) found a coefficient of digestibility of hay by sheep of 56·19 per cent. Popoff (95) demonstrated the formation of methane in the rumen; Zuntz showed in addition that similar putrefactive changes occur in the cæcum and Hofmeister (51) that they are also carried out in the colon.

The bacterial nature of fibre digestion in the rumen and intestine of ruminants was demonstrated by Ellenberger (20) and Scheunert (112, 113), and the nature of the bacterial flora concerned was carefully studied by Ankersmit (2), Hopfie (59), Henneberg (42, 43), Schiebich (121, 122). On the other hand, it was shown by Mangold's work (83, 84), thus disposing of earlier views, that the infusoria of the rumen take no significant part in digestion of fibre. It has been shown by Tappeiner (130, 131) and by Zuntz with Markoff (152, 88) and other collaborators, that the end products of the bacterial fermentation of fibre are gases (CO<sub>2</sub>, CH<sub>4</sub> and H<sub>2</sub>) and acids (acetic, lactic, butyric, isobutyric, valerianic, formic, propionic and succinic). Henneberg and Stohmann (44) assumed the occurrence of soluble intermediary products which Zuntz (153) and Ellenberger (20) conjectured to be dextrins and sugars. Woodman (145) isolated sugar from the products of bacterial fermenta-

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tion of fibre by horse feecs, and showed with Stewart (146, 148) that cellulose is split in the rumen to cellobiose and then to glucose.

The acids arising from further degradation, according to Zuntz (150), are utilised in the metabolism of the runinant. This is true especially, of the fermentation lactic acid (88, 29); Zuntz and Mallevre (79) believe that butyric and acetic acids also serve as sources of energy.

Woodman (147) considers that fermentation acids may have a sparing effect on the combustion of body fat, but regards them as of no significance for fat formation. He is also of opinion (145, 147) that, if utilisation of fermented fibre does not occur through absorption of the sugar formed, the processes occurring in degradation of cellulose by bacteria in the gastrointestinal tract of plant eaters are to be regarded merely as incidental to digestion, and that the acids arising can be utilised only by the bacteria themselves.

Fermentation in the rumen may give rise to different end products. Henneberg and Stohmann showed (44, pp. 52, 389) with bullocks, and this has since been confirmed, that a large addition of easily digestible substances such as molasses, cane sugar or starch, reduces the digestibility of fibre. Zuntz (151) explains this on the grounds that the bacteria ferment instead of cellulose, the more easily attacked starch and sugar, which consequently lose some of their value and are less well utilised. Zuntz (152, 154) with Markoff (88) and other collaborators, also showed that addition of sugar to the contents of the rumen increased fermentation processes threefold but depressed digestion in cattle.

#### COEFFICIENTS OF DIGESTIBILITY BY FARM ANIMALS.

Coefficients of digestibility of fibre for the numerous farm feedingstuffs, as determined with ruminants and other farm animals in many feeding and metabolism experiments cannot here be dealt with in detail. Reference may be made to the tables of Kellner, Armsby, Forbes, Hansson and others.

For the horse, the digestibility of fibre in a ration of oats and chopped hay was found by Zuntz and Lehmann (156) to be 13.85—32.59 per cent. Cellulose is not dissolved in the stomach (117, p. 25), but only in the caecum (52, 110, 111, 114) and colon. The fermentation of cellulose shown by Ellenberger and Hofmeister (21) to occur in the contents of the lower ileum, is insignificant in amount (117). More recent work by Teschner (132), Hotzel (50), Wrede (149) and Engler (22) from the Ehrenberg Institute, shows a digestibility for draught horses of 21.48 per cent. for oat meal; 28.36 for straw; 30-36.8 for hay; 39 for steamed potatoes; 52.40 for potato silage. All values were considerably reduced by simultaneous feeding of molasses.

Digestion of crude fibre in the *pig* does not occur either in the stomach or the small intestine, but only in the caecum and colon (111, 73). Degradation here also is by bacterial action; extracts of mucosa are inactive (111).

With reference to the digestibility of the fibre of different feedingstuffs by pigs, special reference may be made to the extensive investigations of Honcamp (e.g., 57). On account of the wide variations in individual experiments, as shown in those of Mangold and Stotz (87) with rye meal, it is hardly possible to give general averages for the digestibility of fibre in different feedingstuffs. Thus, for instance, for fibre in rye meal the following values have been found: Kellner, 2·3; Lehmann, 10; Honcamp, 19·7 (11·9—27·4); Mangold and Stotz, 25·66 (0·0—36·06) per cent.

The reason for these wide variations in fermentation of fibre in the pig is probably to be found in the changes of intestinal bacteria with the nature of the ration, changes similar to those found in the dog and in man, and in the effect of differences in the physical state of the feed.

#### ENERGY VALUE AND UTILISATION.

From the point of view of the energy value and utilisation of fibre by animals, it is important that fibre is broken down by bacterial digestion to the same end products as are starch and sugar. The stomach and intestinal gases produced by this bacterial fermentation, and also part of the acids, are not utilised in the animal body, and are lost. The same value may be assumed for such part of these products as is absorbed as sugar or fatty acids or used for constructive purposes by the bacteria themselves. In agreement with this view, Kellner (66) in 8 experiments on bullocks with 1 kg. potato starch or straw or cane sugar found weight increases of 248, 253 and 188 g. respectively, indicating an energy utilisation of 56.4 per cent. for starch and 57 per cent. for fibre with fermentation losses of 43.6 per cent, for starch and 43 per cent, for fibre. Kellner found also that the energy value of the digestible part of fibre corresponded fairly closely with that of cellulose and pentosans. For methane, according to Kellner (66, p. 95), 14 per cent, must be deducted, so that the utilisable energy of 1 g. fibre is 3,599 calories. Similarly, Henneberg and Stohmann (45) calculate 1 g. fibre as equivalent to 3,519 calories (cf. also 55).

It has, however, been pointed out by different writers that the doctrine of equal net energy values for fibre and starch in ruminants is, in some way, unsatisfactory. In the first place as Zuntz (150) showed as early as 1879, the digestion of fibre, unlike that of starch, involves work, so that the net energy is the metabolisable energy minus the energy used in digestion. Zuntz (36) estimates the loss in ruminants per 1 g. fibre as 0.14 Cal.; Ustjanzew estimates 0.31 Cal. for chewing

and cudding and 0.5 Cal. for intestinal work, or. altogether 0.64 Cal. (17) or 0.84 Cal. (138), i.e., for example, per kg. hay in ruminants 94.2—111.7 Cal. (68). The loss in horses is higher, 167 Cal., since here the more intensive chewing exceeds the work of rumination and fermentation in the rumen. According to Zuntz, Lehmann (156) and Hagemann (155) the work of chewing and digesting the fibre of a normal ration in horses represents 9 per cent., or for 1 g. fibre, 2.65 Cal. In straw feeding, more energy may be used for digestion than that supplied by the nutrients absorbed (155, p. 280).

In the *pig*, the utilisable value of fibre is lower, compared with starch and sugar, since less of these is lost in fermentation; Fingerling (24) found for 1 kg. starch, 355 g. as compared with 248 g. for fibre, giving a value for 1 g. fibre of 4186.2 cal.

#### OTHER ANIMALS AND MAN.

In the *rabbit* the caecum is again the site of fibre digestion (157, 137); v. Knieriem (69) Konig (70) and Thomas and Pringsheim (134) found a fairly high digestibility of crude fibre, cellulose and pentosans, amounting in carrots to 65.3 and in cabbage leaves to 77.99 per cent.

Experiments by Fredrich (26) working under Lintzel at this Institute, showed that rats can utilise fibre to a considerable extent. White rats on a mixed diet containing 3.3 per cent. crude fibre in wheat and barley meal and potato flakes, showed a coefficient of digestibility of 46.54 per cent., with variations from 38.95 to 51.92 per cent.

Some workers have found crude fibre or cellulose, largely indigestible by the dog (69, 118, 119, 116, 48, 134). Rubner however (101, 103, 104, 106), studying the utilisation by the dog of different additions to a meat diet, estimated the digestibility of the plant cell membrane in three fractions, cellulose, pentosans and residue, and found considerable degrees of digestibility. The coefficient of digestibility of paper was 20—25 per cent., that of birch sawdust 30—40 per cent. and of wheat bran 25 per cent. Waentig (140), using Weende crude fibre from rye straw, found digestibility zero when the fibre was baked in bread, but 9.7 per cent. when added to a mash of potato flakes, while untreated rye straw in bread gave a digestibility of 10 per cent.

Thomas and Pringsheim (134), using filter paper instead of Weende fibre, or pure cellulose prepared by Cross and Bevan's method (16) got completely negative results. They explain the fact that results are sometimes positive and sometimes negative as due to variation in the intestinal flora, the dog evidently being at times without cellulose splitting bacteria. Thus it may be assumed that, although the

digestive system of carnivores is not naturally adapted for plant food, a dog may develop an intestinal flora which makes possible a certain amount of digestion of fibre. The house dog is fed as an omnivorous animal and often with much plant food so that, with this food, it will also ingest cellulose splitting bacteria. Bacterial fermentation in this case will probably attack chiefly the more delicate vegetable celluloses which are also digestible by man. But the fibre of potatoes and cereal grains were found to be largely digestible in experiments of Lossl at this Institute on fox terriers, although there were wide variations in different experiments. Of the Weende fibre, the dogs digested; from barley meal 7.67—33.53; potato flakes, 15.37-23.07; boiled potatoes, 3.27—86.83; rye meal, 14.54-24.28; wheat meal, 2.92—87.52 and rice, 25.24—80.05 per cent.

These wide variations in fibre digestion by the dog appear understandable in the light of the possible differences in bacterial flora in the intestine and the similar observations on the wide individual differences in fibre digestion in man, In man, also, the alimentary tract normally digests cellulose in different degrees depending on its origin, age and hardness; sometimes digestion may be almost complete.

On account of the fact that most investigations have been made on a limited number of subject with only single experiments, and of the large range of variation, it is impossible to give figures for the average digestibility by man of fibre in different foodstuffs. Weiske (143) was the first to study fibre digestion in man, and he found in two subjects, on pre- and post-experimental diet free from cellulose, digestibility coefficients of 47.3 and 62.7 per cent. for carrots, celery and cabbage. v. Knieriem (69) found 25:32 per cent. digestibility for fibre of lettuce. Lohrisch (76) on the basis of a few 1-2 day experiments on 3 subjects, gives the following fibre digestibilities; white cabbage, 100; carrots, 95; spinach, 90; bread, 59-85; split peas, 45 per cent. Rubner carried out numerous single experiments on human subjects in his studies of the digestibility of plant foods, using different kinds of vegetables, fruits and bread. Of the chemical components of the cell membrane, he found in some kinds of fruit and vegetables, that cellulose was 79.06, pantosan, 81 81 and residue, 82 86 per cent. digestible. For different types of bread the corresponding figures were 32.81, 45.46 and 33.26 per cent. The cell membrane of potatoes was particularly easily digested, a characteristic which justifies their wide use as a foodstuff (108).

In the study of the digestion of fibre in man, microscopic examination of the faeces is of particular importance. It is found that the delicate membranes of, for instance, savoy cabbage or carrots (Rubner, 102) are completely digested; those of spinach, with the exception of the lignified fibres of the spiral vessels (35, 47), and those of potatoes also to a great extent (1,129). Well lignified parts and cork are not affected (101).

According to Rubner and Lindner (75) the aleurone cells of cereal grains pass through the human digestive tract without solution of their cell walls and without satisfactory digestion of the cell contents; the same is true of the horse and sparrow (75) and similar observations have been made by Mangold (80) and Kruger (71) on poultry.

Undigested fibre, both by its own bulk and also because of increased intestinal secretion in man and the dog, causes an increase in the volume of faeces (104, 105). This is true in particular, when the bran content of bread is increased (105, 107); the digestibility of fibre and of the pure cellulose and pentoses of bread is reduced, in the case of rye bread from 58.7 to 46.1 per cent. (30, 105, 92). If digestibility is low increase of cellulose results in loss of energy value, since the loss of calories in the faeces is larger than during low cellulose control periods (25).

In general, at least in man, the young non-lignified cell membrane of food plants is much more easily attacked and digested by bacteria than older, lignified structures (49). In man the small intestine shares in this process, as has been shown experimentally by Gosmann and van der Reis (31). These workers found that raw or cooked cellulose can be broken down and rendered soluble, not only by the bacteria from human faeces, as was shown by Khouvine, Delaunay and Hopffe, such bacteria being capable according to Remy (98) of fermenting 42-94 per cent of fibre, but also by bacteria from the colon, and even the upper middle and lower parts of the small intestine, while Berkefeld filtrates are inactive. Since samples taken by means of metal capsules from the empty small intestine were active, these workers are inclined to the view that the human small intestine has an obligate flora of cellulose fermenting bacteria. The part played by the small intestine in digestion of fibre is confirmed by observations of Strauss (129) on patients in whom the large intestine was excluded by operative measures.

With regard to individual differences in digestion of fibre and other factors which influence it, and also with regard to the utilisation of the energy of cellulose, the same general rules apply to man as have already been discussed or will be dealt with later. The rôle of pentosans in human metabolism will be discussed below in the section dealing with these substances.

For a long time it was not recognised that fibre can be digested by poultry (112; 27, page 143). It was commonly regarded as indigestible roughage (Durigen). Mangold has, however, shown (81, 82) that this older view was the result of faulty technique. and, in particular, of too short experimental periods. Further, more detailed experiments of Katayama (65) on poultry, with different feedstuffs, in which faceal and urinary excreta were collected separately by means of an artificial

anus, the abnormal conditions set up prevent the digestibility coefficients obtained from being altogether reliable (cf. also 41,78).

From our knowledge of the nutrition of wild birds, their ability to digest fibre must be regarded as highly probable. Further, the two long and capacious caeca indicate the chief locus of fibre digestion and the nature of the caecal faeces, which are always excreted separately from the intestinal faeces, and in the Scottish grouse (124) as well as in the fowl (139, 81) and the goose (Heinroth), form a pasty, evil smelling mass, gives a similar indication. By comparison of chemical analyses of caecal and intestinal faeces, and by metabolism experiments on normal fowls and fowls after operative removal of the caeca, Mangold (81) with Roseler (99) and Radeff (97) showed that the caeca are the chief sites of fibre digestion, and this was confirmed with Meyer (90) by microscopic examination of the caecal contents. Extending these studies, Mangold and his collaborators, Henning (46), Bruggemann (11), Stotz (128) and Peters (94) in this Institute, determined the coefficient of digestibility for fowls of fibre in many feedstuffs, using a modification of the Weende method. In these studies the new method of Stotz (127) was used for the chemical separation of urine from the faeces excreted with it.

From these investigations it appears that the digestibility of fibre by poultry is so high for many feedstuffs that it must be taken into account in calculating the value of a ration. For barley, maize, oats, peas and cabbage, digestibility coefficients for fowls, pigeons, geese and ducks of over 20 per cent., and for maize and barely as high as over 30 per cent. were found.

It also appeared, however, that the digestibility of fibre in different varieties of the same cereal varied greatly. In the experiments of Stotz and Bruggemann (128), who in each experiment used several birds, usually Rhode Island hens, the differences between different varieties were so great that the values varied, e.g., for barley, between 0 and 31.5 per cent. For oats, Halnan (37) also found a variation in fibre digestibility by Leghorns from 0-6.6 per cent. Errors in technique can scarcely be responsible for these results since the experiments were carried out with the same methods in the same Institute. It should also be noted that other nutritive constituent of the ten varieties of barley studied by Stotz and Bruggemann showed a general uniformity in their coefficients of digestibility.

It may therefore be taken as true in general of the digestibility of fibre in feeds-tuffs and foods that even with the same species of plant, the variety and origin, in addition to all other factors, are of importance. The reason of this is probably to be found in varietal differences in chemical and structural make-up of the cell membrane. This is probably true not only for the cereals studied, but also for different varieties of the same vegetables and fruits, of importance in human diet.

The supplementary observation by Mangold and Schaff (109) may be mentioned, that, of other Sauropsida, the tortoise has a very considerable power of digesting fibre. For Testudo graeca the average digestibility of fibre in barley was 10; in white maize, 30; hay, 45; yellow maize, 71; lettuce, 84; and in carrots, 93 per cent. The locus of fibre digestion here also was the caecum and proximal colon. The long time taken for food to pass through the gastro-intestinal canal, in Testudo 24 days, and up to 38 and even 55 days for the last traces, greatly facilitates the action of bacteria on the fibre.

#### PENTOSANS.

Of the substances closely associated with orthocellulose in the cell membrane and in fibre, the hemicelluloses, including pentosans, are of most importance. These polysacharides are also acted on by bacteria (122, p. 496) and fermented by those of the rumen (8, 9). According to Kellner (66, p. 162) pentosans promote fat formation. Experiments of Iwata (61) and of Furth and Engel (28) on rabbits show that xylan, a pure pentosan, can be broken down in the organism and converted into reducing sugar.

According to Rubner (101) pentosans are food constituents of importance for plant eaters. In the sheep, Stone and Jones (126) found pentosans of grass and bran 49-90 per cent. digestible; Konig (70) found those of grass hay 82-06, of clover hay, cut before flowering 76-41 and of pea straw 44-02 per cent. digestible. Thomas and Pringsheim (134) found those of potatoes 72-79 per cent. digestible.

In man, pentosans are of less importance, since only a few grams of pentoses and pentosans are ingested daily. Grafe (32, 33) carried out experiments with xylose of which quantities up to 30 g. daily were well tolerated. It appeared that in man as in the dog, individual variations occurred, from 20-60 per cent. being excreted in the urine; and that pentose metabolism is largely independent of dextrose metabolism, the increase in blood sugar after xylose being wholly as pentoses and not as dextrose. Respiration experiments, however, showed that the xylose that was not excreted was burned, and had a protein sparing action.

#### PECTINS AND LIGNIN.

Pectins also, which act as intercellular cementing substances, are chiefly acted on by bacteria in the animal organism (43, 120, 121, 122); such bacteria were shown by Ankersmit (2) to occur in the digestive tract of the horse and, by means of the pectinase, they produce, to separate the plant cells and make the digestion of cellulose possible.

Lignin is the most resistant part of the cell membrane, and is regarded as, in general, non-digestible (44, 77), although Konig (70) gives the digestibility of lignin by

sheep as 16-23 per cent. in hay, 4-13 in clover hay, and 28 in pea straw, the corresponding figures for cutin being 7·27, 10·67 and 20·93 per cent. while those for pure cellulose, pentosans and total fibre varied between 42 and 83 per cent. According to Henneberg and Stohmann (44) and Dietrich and Konig (18) the non-digestible residue of fibre corresponds to the lignin content. Konig considers that the utilisation of fibre and of the organic constituents of plant feedstuffs as a whole depends on the content of lignin and cutin, but Rubner (106) and Woodman and Stewart (148) have shown that the digestibility of cellulose does not depend only on the degree of lignification or the lignin content, since, for instance, in grass, the decrease in digestibility with age is associated with only a relatively small increase in lignin values.

According to Rubner (106), of the small quantity of lignin ingested daily in bread by a human experimental subject, about half was digested. Thomas (133) on the other hand considered that the apparent partial digestion of a lignin preparation depended on the presence of cellulose. Experiments of Rogozinski and Starzewska (100) on ruminants with pure lignin or lignin in straw, gave completely negative results.

Cork substance, suberin, is also regarded as non-digestible (44).

#### FACTORS AFFECTING DIGESTIBILITY.

Certain observations on the effect of different factors on digestion of fibre may be briefly summarised. Reference has already been made to the influence of individuality. What has been said is equally true for animals and man. Henneberg and Stohmann (44, p. 451), recognised the importance of individuality and of nutritional condition. Kellner (66, p. 49) considered that the most important reasons for poor utilisation of food lay in faulty dentition, too rapid eating or pathological conditions. Individual differences in capacity to digest fibre should, nevertheless, be regarded as fundamentally physiological, for every test of digestibility of feedstuffs in animals of the same species and origin shows that they are organisms differing in fine details of constitution. In studies on digestion and food value, this fact is not sufficiently considered.

It would take us too far to attempt to define the range of variation due to individuality from the coefficients of digestibility found by different workers in studies on digestion and utilisation of fibre. But such individual variations occur throughout.

In man, Rubner and Thomas (106, 107) have shown that, for instance, cellulose may be digested to different degrees by two experimental subjects while pentosans are equally well digested; that, in fact, the digestibility of fibre in man is ex-

tremely variable and consequently the experimental results of different workers on e.g., the digestibility of different kinds of bread, can scarcely be compared and used to give averages valid for the general case. These differences are considered to be due to individual differences in intestinal flora, and the environmental conditions of the bacteria in the intestine. Jansen and Muller (60) have made similar observations on fibre digestion in 5 experimental subjects who gave coefficients varying between 79·1 and 90·0 per cent. for wheat bread with a low fibre basal diet, and between 58 and 75 per cent. for potatoes.

The variability of results for fibre digestion is probably also affected to a significant degree by the nature of the rest of the food and of the total diet fed. This will have an effect on the intestinal flora, altering the culture medium for the bacteria (101, 133, 134).

These factors must be of special importance in ruminants, in which, on account of the large quantities of fibre to be dealt with, the possibility of such quantitative changes is greater. We have already referred to the depression of fibre fermentation in the rumen due to simultaneous teeding of easily soluble carbohydrates. It has been shown by Henneberg and Stohmann (44), that this effect on fibre utilisation is bound up with the nutritive ratio (protein to other nutritive constituents); this has recently been confirmed by Mertins (89), who found in sheep that addition of protein to the ration decreases fibre digestibility.

Similar effects of changes in the composition of the ration occur, according to Rubner (103) in man and the dog, and according to Bollmann (6) in the pig.

It seems likely that the degree of mechanical division of the food would have an effect on the utilisation of fibre and would be of practical importance in man and animals. In contrast to protein, fat and starch, which can be attacked and rendered soluble at once by the digestive enzymes, it seems natural to expect that the chemical digestion of fibre, depending as it does on the action of bacteria, would be affected by an increase in the surface area, open to attack by the bacteria, by mechanical subdivision of the food. It is true that machanical treatment in the animal body takes place in chewing, rumination and grinding in the gizzard of poultry. But, since the work of chewing consumes a part of the tood energy and so reduces the food value (see above), it is of importance to know whether breaking up the food before ingestion, so as to reduce the work of chewing, would increase digestibility and utilisation of fibre.

Haberlandt and Zuntz (36, p. 707) in their experiments with birch wood meal, found that the grinding of the wood, which they adopted to secure availability of the cell contents, served also to make available the cell walls. The digestibility of

the fibre is, however, not necessarily increased in proportion to the degree of subdivision. Kellner (66, p. 278) both with wheat and barley straw in cattle, found no difference whether the straw was fed chopped or as meal. In the horse, however, Gay (cf. Kellner 66, p. 279) found an increase in digestion of fibre from whole oats 42.0 per cent.) to ground (48.9 per cent.) and crushed grain (63.6 per cent.). In pigs the advantage of milling has frequently been observed. Thus Haberhauffe (34) working with maize, found a fibre digestibility of 52.39 per cent. with whole grain, 55.66 per cent. with coarsely ground, 75.86 with medium ground and 78.05 per cent. with finely ground grain, while Honeamp, Schram and Stotz (58) found a higher starch equivalent for oatmeal than for whole oats; Woodman an increase of 15 per cent. in the feed value of ground as compared with whole barley, and Nils Hansson (38, 39) an increase in feed value of maize of 5-6 per cent. by crushing, 10-12 per cent. by coarse grinding and 15-18 per cent. by fine grinding.

Similar observations have been made on fibre digestion in man.

In addition, numerous other factors affect the digestion of fibre, above all, differences in structural and chemical composition of the cell membrane as determind by variety, origin, age and conditions of growth of plants; the effect of drying (grass and hay), the temperature used (cf. Watson and Ferguson, 142), ensiling, cooking boiling and so on. Although these factors are of greater importance in the feeding of animals than of man, yet in human nutrition methods of preparation of plant food will have a similar action in modifying the digestion and utilisation of fibre as well as on the availability of cell contents.

In conclusion, reference may be made to the numerous attempts which have been made to treat fibre in straw and wood so as to increase digestibility and utilisation of types which are otherwise non-digestible or only slightly digestible. It may be recalled that both with straw, treated with alkalis by the method of Lehmann and others (cf. 66, 23, 53), or without chemicals (84, 54, 56), and also with wood quite apart from conversion into sugar as proposed by Bergius), treated by mechanical and chemical means (35, 36), or by the more recent colloid chemical methods of Schwalbe (123) and Mangold (86), feedstuffs may be prepared with greatly increased fibre digestibility, comparable with that of other feedstuffs. But, since the questions involved in these process are chiefly technical problems of treatment and preparation, there is no need to discuss here the various methods used and the results of testing the products as feedstuffs.

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# EFFECT OF CALCIUM-DEFICIENT ROUGHAGES UPON MILK YIELD AND BONE STRENGTH IN CATTLE.

BY

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#### INTRODUCTION.

Dairy cows depend upon roughages as the principal natural sources of calcium, and upon grains and milling by-products for most of the phosphorus. Grasses provide from one-fifth to one-third as much calcium, and about three-fifths as much phosphorus as do the legumes grown under the same conditions. The calcium content of the forages grown on low-lime soils is lower than that of the same kinds of plants on more fertile soils. Lactating cows dependent on grass forages grown on low-lime soils often deplete their reserves of calcium to such an extent that skeletal strength and yield of milk are affected.

#### STATEMENT OF PROBLEM.

The Jersey cows in the Florida Agricultural Experiment Station dairy herd were dependent upon pasture grasses, and corn or sorghum silages, as the main sources of roughages. Since December, 1920, alfalfa meal was included as one-ninth to one-eighth of the concentrates mentioned in the herd records and annual reports of the station. One cow—No. 18—received alfalfa meal prior to that time, while she was on Register of Merit test.

All rations mentioned during this time contained an adequate proportion of protein, total digestible nutrients and phosphorus for cows of their weight and milk production. Under the conditions of feeding just mentioned, these Jerseys had an average milk yield of approximately 4,000 pounds per year. A number of them suffered broken bones—hips, ribs and even a pelvis—under this general feeding practice.

The rations were changed in January, 1929, by the addition of two per cent. of bonemeal to the mixed concentrates, based upon a study of the conditions noted. Bonemeal was chosen in preference to a local supply of calcium carbonate because the former was known to be free from fluorine and other undesirable substances. This proportion of mineral supplement was assumed to supply sufficient calcium to provide for maintenance, a reasonable milk production, and to allow a surplus with which the cows could restore the body reserves.

#### REVIEW OF LITERATURE.

Eckles (2) observed that cows on phosphorus-deficient rations produced much less milk than when the lacking mineral was supplied. Theiler (7) and Tuff (8) reported similar conditions among cattle in parts of the Union of South Africa and of Norway. Fingerling (3) obtained similar results with milk goats on rations the basal portion of which was deficient in calcium. Meigs and Woodward (5) concluded that cows produced appreciably more milk after having been placed in good mineral storage during the preceding dry period.

Kellner (4) mentioned that dairy rations are deficient in calcium more frequently than they are in phosphorus. Under these conditions, cows withdraw mineral matter from the bones in order to maintain milk production. As this impoverishment progresses, the milk yield is decreased.

Nessler (6) found that the bones of a cow affected with *Krochenbruchigkeit* (bone-fragility) contained less calcium and phosphorus, were lighter in weight, and had thinner shaft walls than had those of a healthy cow. The spongy bones—vertebrae, pelvis and joints—were affected more than the shaft bones of the legs. This depletion of calcium and phosphorus in the skeleton occurred when the cattle received feeds inadequate in the bone-forming mineral elements.

#### PLAN OF INVESTIGATION.

Typical rations used with the dairy herd were assembled from the herd records and annual station reports, and a study made of them. The rations were changed slightly in January, 1929, by adding two per cent. of finely ground feeding bonemeal to the concentrates. Some of the higher producing cows were given a daily allowance of about five pounds of alfalfa hay. Some changes were made in the proportion of concentrates based on local market prices of nutrients, and upon availability of these feeds.

Twelve Jersey cows in the herd had completed lactations on the unsupplemented rations, and also upon the supplemented rations after January, 1929. Their lactation records were assembled by 10-day periods, computed to maximum age basis, using the factors of Clark (1) for Jersey cows milked twice daily not under official test conditions. An average lactation curve was computed, weighted on the "per cow" basis.

Cow No. 59 broke her pelvis late in December, 1928. Following the autopsy, breaking strengths were determined upon her femurs and humeri, using an electrically driven Riehle Brothers testing machine, and applying the weight slowly from above in the middle of 7- and 5-inch spans of the shafts. After bonemeal had been added to the rations, leg bones were obtained from additional Jersey cows as they

were eliminated from the station herd, and bone strengths determined similarly, using a 6-inch span. Similar data were obtained from the leg bones of three range cows, six Aberdeen Angus cows and 24 three-year old steers. The steers had free access to bonemeal during one year prior to slaughter, while the Angus cows received a limited grain allowance which contained one per cent. of bonemeal. Both the steers and Angus cows received pasture grasses and silages grown on the same types of soil as that fed to the Jerseys.

#### PRESENTATION OF DATA.

The data presented herein were accumulated under the conditions of management prevailing over a period of years in the station dairy herd generally, rather than from an investigation planned in advance with a limited number of selected cows. Detailed search of the herd records and experiment station reports showed that pasture grasses (largely Bahia, centipede and Bermuda grasses), corn, cane or sorghum silages were the principal roughages in use. Summation of published data from seven early feeding trials between 1908 and 1917 showed that Jersey cows with an average weight of 711 pounds and a 15 pound milk yield daily, received 22\frac{1}{4} pounds of silage. Their concentrates were mainly one part of cottonseed meal (36 per cent. protein) with 2 to 4.4 parts of wheat bran. Other concentrates fed at times included cocoanut meal, wheat middlings, velvet beans in the pods or ground into feed meal. One test cow ration consisted of 300 wheat bran, 200 velvet bean feed meal, 100 commeal and 80 parts of peanut meal (grade not stated). Alfalfa meal was mentioned first in the ration of Cow No. 18 on Register of Merit test, and again in December, 1920, when it became one-ninth to one-eighth of the regular concentrate mixtures fed. During two feeding trials of 123 and 84 days, 4 and 6 cows respectively received not in excess of four pounds of alfalfa hay daily. Mineral supplements, except common salt, were not used until shortly prior to 1929, and then for but a limited time.

The concentrate mixtures used more recently in connection with the roughages mentioned are listed in table I. Cost and availability of oats, corn gluten feed and peanut meal (44 per cent. protein) caused them to be replaced with locally grown velvet bean feed meal, higher grade cottonseed meal and by changing the proportions of the feeds used.

Calcium and phosphorus contents of the several typical rations were calculated, using average analyses of purchased concentrates, and analyses of the locally-grown feeds. All of these rations provided an adequate supply of protein and total digestible nutrients, and exceeded the requirements given by Kellner (4) and Wellman (9) for phosphorus. The supply of calcium was noticeably inadequate.

Table I.

Concentrates used with silages and grass pasture in feeding the Florida Station dairy
herd from 1922 to 1933.

Year	1922 1923		1924 and 1925	1928	1929 to 1933		
Ration		Ration 1	Ration 2	Ration 3	Ration 4	Ration 5	
Wheat bran Cornmeal	r cent.	100 100 75 50 50 50 	100 100 75 50  100 	100 100 100 50 	200 300 300 300  100 100 100 100	400 300   100  200 100	
Velvet bean feed meal Common salt Bonemeal		•••	•••	 	***	200 13 26	

The concentrates in general use since January, 1929, containing two per cent. of bonemeal, provided calcium sufficient to meet the requirements for maintenance, a more liberal milk yield, and to allow mineral reserves to be restored to the skeleton. No cow in milk in the station herd has had a broken bone since that time, and the milk yield has increased, as will be shown. A comparison of the amounts of calcium and phosphorus contained in these several rations is shown in table II.

Lactation records were assembled and tabulated for the 12 Jersey cows that had completed lactations both on the earlier low-calcium rations with the concentrates listed in table I, and on the rations containing bonemeal. A total of 44 lactations were included in the low-calcium group. The average actual yield per lactation, weighted on the "per cow" basis, was 3,980 pounds of milk, or an average of 13.38 pounds daily during these 44 lactations of 400 days or less. These same 12 cows completed 22 lactations on the high-calcium rations, averaging 6,425 pounds of milk, or 17.55 pounds daily. The earlier group of lactations averaged 297 days in length, in contrast to 366 days for the latter group.

There was a marked tendency for these cows to attain a higher maximum daily milk yield, and to decline in milk flow less rapidly when the low-calcium rations had been supplemented with bonemeal.

Since these lactations were begun at varying ages, they have been computed to a uniform maximum age basis, using the factors obtained by Clark (1) with Jersey cows milked twice daily not on official test. The lactation curves so computed, are

TABLE II.

Comparison of the nutrients required by a typical cow in the station dairy herd and of the nutrients provided by rations in use between 1908 and 1933.

	Rat	Rations		Nutrients provided					
	Corn silage	Concen- trates	Digestible crude protien	Total digestible nutrients	Calcium (Ca)	Phosphorus (P)			
	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.			
Requirements— 725 lb. cow yieldin 15 lbs. of 5 per cen milk.*		•••	1.5050	11.4756	0·0800—·1059 ·0789‡	0·0394—·0458† ·0323‡			
Rations in use— 1908-1917 . 1922-1925 . 1928 . 1929-1933 .	. 22·25 . 22·25 . 22·25 . 22·25	12·0 11·0 10·5 11·0	2·3178 1·907 1·8733 1·6811	11.6633 11.638 11.6600 11.6075	·0306 ·0448 ·0408 ·0776	·1705 ·1973 ·1535 ·1032			

<sup>\*</sup> The average requirements are based on weights and milk records of cows in feeding trials conducted between 1908 and 1917 at this station.

<sup>‡</sup> Calcium and phosphorus requirements calculated according to Kellner's (4) requirements.

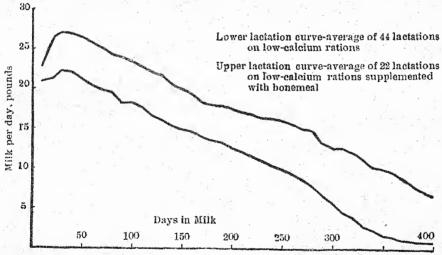


Fig. 1. Average daily milk yields of 12 Jersey cows before and during use of bonemeal as a supplement to low-calcium rations.

shown in figure 1. These milk records were assembled by 30-day periods, and the relative rates of milk yields calculated in relation to the milk yield on the unsupple-

<sup>†</sup> Calcium and phosphorus requirements calculated according to Wellman's (9) requirements.

174 THE INDIAN JOURNAL OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY [ IV, II. mented rations. This comparison of monthly milk yields on the low-calcium and on the supplemented rations is shown in table III.

Table III.

Persistence of milk production of 12 Jersey cows as affected by addition of bonemeal as a supplement to low-calcium rutions.

*	Production or rat	n low-calcium ions	Production on supplemented rations			
Month	Milk yield	Rate of pro- duction*	Milk yield	Rate of base production*		
	Lbs.	per cent.	Lbs.	per cent.		
1	644.3	13.84	765-1	16:43		
2	636.2	13.66	789.9	16.96		
3	572:1	12.29	732·1	15.72		
4	525.5	11:29	681.3	14.63		
5	464.0	9.96	622-2	13:36		
6	420.6	9.03	553.2	11.88		
7	378.2	8.13	525.3	11.28		
8	328.2	7.05	493.7	10.60		
9	281.2	6.04	464.1	9.97		
10	201.9	4.34	406•7	8.73		
и	112.6	2.42	359.0	7.71		
12	55.4	1.19	295.4	6.34		
13	27.6	•59	237.7	5.11		
10 days	8.4	•18	66.2	1.42		
Total .	4,656-2	100.00	6,991.9	150·16		

<sup>\*</sup> The total production in the first lactation curve (4,656.2 pounds of milk) is the base figure used in the computing the rate of production. These lactation curves are weighted on the "per cow" basis, computed to the maximum age by using the factors of Clark (1), so as to place individual lactations upon a comparable basis.

Over fifty per cent. more milk was produced per lactation of 400 days or less on the supplemented rations. These 12 cows yielded 31:17 per cent. more milk per day for each day in milk (under 400 days) during these lactations. This latter point takes into consideration the fact that the cows tended to milk over longer lactation periods on the supplemented rations.

#### SKELETAL STUDIES.

Five cows in a herd of 34 on the low calcium diet had suffered one or more broken bones, as follows:—

No. 59—pelvis broken in three places.

No. 120-both hips and right 12th rib broken.

No. 223-right 13th rib.

No. 225-left hip and last four ribs on right side.

No. 229—left hip.

Even a 10-year old bull—Florida's Majesty 153431—was slaughtered in 1927 because of a broken hip that affected his usefulness. This large proportion of animals with broken bones called attention to the extremity of the condition caused by the low-calcium rations. Cow No. 59 went down with a broken pelvis during late December, 1928. Upon autopsy, the femure and humeri were obtained, and average breaking strengths of only 335 pounds were recorded. In these determinations, 7- and 5-inch spans respectively were used, but in all subsequent studies of bone strengths, a standard 6-inch span was adopted. As more cows were eliminated from the herd, the long bones of the legs were obtained, and their strengths determined.

It is of interest to note that the femurs and humeri of four Jersey cows receiving the supplemented ration for the last 19 to 27 months, averaging over 10 times as strong as the corresponding bones taken from Cow No. 59. In every instance the femurs and humeri exceeded the cannon bones in average strength. Noting this relationship, it was possible to use strengths of cannon bones as indices of the state of mineral storage of the cows.

The strengths of No. 59's leg bones represent an extreme stage of depletion of mineral reserves in the skeleton. This cow had been a persistent producer, averaging 6,338'6 pounds of milk in her 11 lactations, and in the last lactation produced 8,159'9 pounds of milk in 531 days. That this cow is not an isolated instance is suggested by the proportion of cows in the station herd at that time that had broken one or more bones.

The strengths of cannon bones from 24 three-year old steers represent a medium stage of mineral storage in the skeletal tissues. Three mature (dry) range cows for which bone strengths are presented, were in a lower stage of storage. On the other

hand, the bone strengths of the dairy cows that had received the supplemented rations indicate that it may be possible for cows to attain a stage of excellent mineral storage, available for subsequent lactations. A summary of these bone strengths, as determined on selected bones taken from cattle under the various conditions outlined, are presented in table IV.

#### DISCUSSION OF RESULTS.

The cows considered in this study received rations that were adequate, considered from the usual feeding standards. This was evidenced by the fact that many of the cows were extremely fat, and yet were not yielding milk in proportion to the intake of digestible nutrients—in many instances they had one pound or more of grain for each two pounds of milk produced. The quality of protein was probably satisfactory, since it was derived from six plant sources—maize, wheat, flax, velvet beans, cotton and sugar beets—in addition to the variety of pasture grasses. The supply of phosphorus from the concentrates was in excess of the requirements. The calcium intake in the average ration was extremely low, even for cows with the level of milk production of Jerseys in commercial dairies.

The depression of milk production was less marked than has been observed in long-continued phosphorus deficiency (2).

TABLE IV.

Average breaking strength of 215 leg bones from Florida cattle under different feeding conditions\*.

	* * *	Average breaking strength of bones from left and right legs							
Cattle	Type of ration	Hume- rus	Femur	Radius and ulna	Tibia and fibula	Fore cannon	Rear cannon	Average	
		No Bo	nemeal av	ailable.				Program (continue) transport (	
Jersey.		Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	
Cow No. 59 .	Dairy ration, unsupplement- ed.	330(a)	340(b)	•••	1,7 1,14 1,14 1,14 1,14 1,14 1,14 1,14 1		•••	335	
Range Cows.		-				, -		- 1	
No. 1	Range on sand and muck lands.	•••		•••		1,530	1,998	1,764	
No. 2	Range on sandy	2,240	2,405	1,923	2,602	1,883	2,155	2,201	
No. 3	Range, peanut hay in winter.	<b></b>	•••	•••		2,120	2,380	2,250	

<sup>\*</sup> Breaking strengths were determined by using a 6-inch span with weight applied slowly in the middle from above, except (a) a 5-inch span, and (b) a 7-inch span with Cow No. 59.

TABLE IV—contd.

Average breaking strength of 215 leg bones from Florida cuttle under different feeding conditions\*—contd.

		Average breaking strength of bones from left and right						
Cattle	Type of ration	Hume- rus	Femur	Radius and ulna	Tibia and fibula	Fore cannon	Rear	Average
	Two	per cent	of bonemee	al in conce	entrates.		<u> </u>	-
		Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.
Guernsey.		-		1 -			- 14	- 1
Cow No. 297 .	Dairy ration, bonemeal I mo.	•••	···		***	3,470	3,440	3,455
Dutch Belted Cow.	Dairy ration, bonemeal 29 mo.	3,535	3,645	3,710	3,240	3,255	3,868	3,542
Jersey Cows.	,			<u> </u>			9.00	
No. 81, 120, 195, 225.	Dairy ration, bonemeal 19— 27 mo.	3,244	3,636†	2,848	3,299	2,228	3,302	3,037
No. 177, 188, 218.	Dairy ration, bonemeal 13— 23 mo.	* •••	•••	•••	3 <b></b>	2,700	3,788	3,244
		3		* * *	I			
	One p	er cent	of bonemeu	in concer	itrates.	*		
Aberdeen Angus		× -	1	. 1	. 1		1	
6 cows	Grass, silage, concentrates.	***-	02). 8 <u>.</u>	***. *	• • •	2,689	3,370	3,030
14 J. S.	, , ,		*. }			*		
Steers.	A	ccess to	bone meal	ad libitum		o i		
8 grade Angus	Grass, pasture, with peanut hay in winter.				***	2,059	3,059	2,559
16 native and grade Here- fords.	Grass pasture, with peanut hay in winter.		•••	•••		2,426	3,184	2,805

<sup>\*</sup> Breaking strengths were determined by using a 6-inch span with weight applied slowly in the middle from above, except (a) a 5-inch span, and (b) a 7-inch span with Cow No. 59.

<sup>†</sup> One femur of Cow No. 195 not available.

Under this condition of inadequate calcium intake, not only were milk yields less than expected, but mineral reserves were depleted to the point of skeletal fragility. A significant proportion of the cows had broken ribs or hips. Since the correction of the calcium deficiency by the addition of bonemeal to the rations, the cows has suffered no broken bones in the next four years, mineral reserves (as measured by bone strengths of cows slaughtered) have been restored, and milk yields have been attained commensurate with inheritance and the organic nutrient intake of the same cows.

#### STATISTICAL ANALYSIS.

Study of the records for possible contributing factors arising from management of the cows disclosed that the dry periods prior to lactation averaged 80 days in length preceding use of bonemeal, as against 86 days while bonemeal was available. This excludes the 12 lactations after the first parturition of each cow in the former period, and one instance of difficult conception among the 22 lactations in the latter period. The average date of conception was at 105 days after parturition in the former interval, as against 169 days (including five cases of difficult conception of 300 days or over) in the latter period. In addition, cystic ovaries were encountered in two old cows, and four failed to conceive. The observation of Dr. C. H. Eckles in Minnesota Station Bulletin 258, was borne out in that the older cows conceived less readily.

From statistical analysis of differences in rate of decline between milk production on the low-calcium rations, and that on the supplemented rations, it was found that the standard error of difference, divided into the difference is 1.73, or that the probabilities are 9 in 10 times that the differences are not due to chance. In other words, these differences are due to inherent differences in the rate of production rather than to chance. This took into consideration the entire lactation curves from the second to thirteenth months inclusive.

The entire lactations include the period immediately after calving, during which the mineral matter stored in the skeleton in the dry period was available. After this available supply is depleted by lactation, the cows become dependent upon the limited amounts in the feed, and milk secretion is checked. When the curves for the 7th to 13th months were analyzed similarly, the factor 72·17 was found in place of 1·73. In other words, it is highly significant that these differences are inherent, rather than due to chance.

#### SUMMARY AND CONCLUSIONS.

Typical rations used in feeding the Jersey cows at the Florida Agricultural Experiment Station during a period of years supplied an excess of protein, energy,

and phosphorus, but were low in content of calcium. Addition of bonemeal as two per cent. of the concentrates was sufficient to render the calcium level adequate for a commercial dairy herd.

This increase in calcium level in the rations allowed 12 Jersey cows to attain an increase of four pounds of milk per day in subsequent lactations, and to be more persistent producers throughout longer lactation periods. At the same time, these cows attained a stage of mineral storage in the skeletal tissues such that the leg bones from nine of them had average breaking strengths in excess of 3,000 pounds. On the other hand, in absence of the calcium supplement, several of these same cows previously had withdrawn mineral reserves from the skeleton to such an extent that an unusual proportion of them had suffered broken hips and ribs.

The possibility of corn silage, grown on low calcium soils, as a source of roughage for use in studies of calcium metabolism is suggested.

#### ACKNOWLEDGMENTS.

The lactation periods prior to May 15, 1928, were accumulated under the direction of Professor John M. Scott, formerly Animal Industrialist and Vice-Director of the Florida Agricultural Experiment Station. Alex R. Mathers assisted in assembling and tabulating the milk records. Professor C. H. Willoughby allowed us to obtain leg bones from Aberdeen Angus cows for use in this study. Professor Charles C. Brown of the Civil Engineering Department made available such equipment as was used in bone strength determinations. Bradford Knapp, Jr., and Dr. L. W. Gaddum assisted with the statistical analyses, testing the significance of the data presented herein.

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#### VETERINARIANS AS 'OFFICERS OF ANIMAL HEALTH'.

[Reprinted from the Veterinary Record No. 8, Vol. XIV, February 1934.]

In the course of an address delivered at the Thirteenth Annual Convention of the Canadian Society of Technical Agriculturists, and reproduced in the February issue of *The Journal of the Ministry of Agriculture*, Sir Daniel Hall, Chief Scientific Adviser to the Ministry, stated that the question of animal disease had been the subject most pressed upon the Agricultural Research Council in Great Britain. It was the one that, to most people, seemed to require the most strenous effort, and many people considered that the most pressing of all the problems before them.

'I am bound to say', continued Sir Daniel, 'while reporting this as the general opinion, I do not personally altogether agree, because I am looking at the problem from the point of view of health rather than of disease. I see the task of the people who are dealing with the health side of animals to be, in future, very much more hygiene and the maintenance of health than the cure of disease. What I would like to see is a class of veterinarians who are officers of animal health rather than practitioners. There must always be practitioners who are concerned with surgical cases and with specific illnesses of valuable animals, but it seems to me that the great efforts of the profession should be rather of a public nature. Instead of being called into this ailing cow, or that fretting horse, we want to see a class of men who have charge of a district, who are thinking about the horses, the cattle, the sheep and the pigs and how to keep disease away from them. Naturally, they will have to know about the endemic diseases, but breeding, environment, nutrition, and other factors in hygiene will be equally important. I think that is going to be the direction in which the veterinary profession itself will eventually move, and that the veterinarian of the future will be the kind of public officer who is taking prophylactic and preventive measures and who is studying problems like nutrition, and so forth, so as to ensure a greater amount of health amongst the animal population. It is only latterly that we have really begun to appreciate what an enormous factor nutrition is in the health of livestock; we do not even yet know all that is necessary'.



## **ABSTRACTS**

Studies on bovine mastitis—VIII. The control of chronic Streptococcus mastitis.

Minett, F. C., Stableforth, A. W., and Edwards, S. J. J. Comp. Path. and Ther. 46, 131-138 (1933).

Previous papers in this series of publications have treated of the bacteriology of Streptococcus mastitis and of the diagnosis of the prevalent chronic form of this disease. The organisms have been grouped as I, II and III of Mustitis Streptococci, the group I being far more responsible for the outbreak of the chronic Streptococcus mastitis and, important from the economic point of view.

Observations covering a period of five years were made in a practically self-centained herd consisting of 53 heifers, dry and milch cows. In the first year of observations, 10 cows were affected with chronic Streptococcus mastitis. During the first two years, preventive measures were adopted and the infected cows were milked last and subsequently kept on separate premises. This led to the formation of a group of cows, which for three years and six months remained free from Streptococci responsible for the chronic and contagious form of Mastitis. Of the ten originally infected cows which remained on the farm for varying periods up to five years, five were under observation for the longest time and they appeared to have made a complete recovery.

The authors conclude that this is the first time that it has been shown by frequent tests of milk that a herd of cows was maintained free from infection, and they prove that chronic Streptococcus mastitis is a contagious disease preventable by the hygienic measure described above.

The Technique used in the examination of milk samples is described in this article. [B, S.]

Russian methods of artificial insemination multiply sires' value. Landauer, W. J. Hered, 24, 87-92, (1933).

The author describes the following methods used in Russia for artificial insemination, and gives photographs of instruments and breeding rack used in this connection. In Russia the need of artificial insemination areas chiefly on account of the great distances in rural districts which frequently made it impossible to use valuable breeding animals to best advantage, and the author thinks it probable that artificial insemination will, in future, play an important part in the breeding programme of stockowners.

I. The Sponge Method of Ivanov was extensively used in the past, but on account of certain fundamental defects it has been discontinued.

II. The Sperm-Collector Method.—Russian experience has shown that this method of getting the sperm with Sperm collector is the most successful one and it has been adopted in Russia for all occasions where large number of ewes are to be inseminated. About 10 times as much sperm can be obtained with the sperm collector and the motility and length of life is also much greater as with the sponge method. In 1931 several hundred thousand sheep, about 187,000 cows, and also horses and donkeys were successfully inseminated by the method. The sperm collector, after being passed into alcohol (65 per cent.) for ten minutes, is, for a short while, put into alkaline physiological salt solution. It is then introduced into the vagina with the help of a pair of pincers. After the ewe has been served, the sperm collector is removed and sperm stored in a glass container. With the aid of a syringe and vaginal speculum, a quantity from 0.2 to 0.4 c.c. of sperm is introduced into the uterus of an ewe. All the manipulations of obtaining the sperm and inseminating the ewes are to be carried out in a room with a temperature between 15-25° C.

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III. The Speculum Method.—In this method sperm is collected with the speculum into a watch glass and used as above. The method is useful if only a few ewes are to be inseminated.

IV. The Artificial Vagina Method.—This method gives the further advantage of dilution of sperm. Good results have been obtained with the dilution fluid containing mucin in case of sheep, while for cattle the dilution in peptone-lipoid solution proved successful. It is found that the sperm of a single ram and bull was sufficient to inseminate 300 to 400 ewes and 1,250 cows respectively during one season. The author points out that at present this method is chiefly employed for experimental purposes.

In a special glucose reagent at a temperature of 10-15° C. sperms are stored successfully for 18 days. [B. S.]

Infectious sterility in the larger domesticated animals. Edwards, J. T. Proc. Roy. Soc. Med. 26, 1192-1210, (1933).

Sterility has been classified under the following headings:-

I .- Genetic.

 $\text{II.--Functional} \quad \cdot \begin{cases} \text{Endocrinal.} \\ \text{Nutritional.} \end{cases}$ 

III .- Infectious.

#### (I) EQUINE STERILITY.

In the equine species a very high percentage of sterility is said to be associated with endometritis in the female. The bacterial content of uterine secretions taken from 1 to 21 days post-partum have been recorded as B. abortus equi, B. Coli, Diplo-streptococci, B. Viscosum equi, and a small percentage of miscellaneous organisms. Most of these organisms have, however, been found as a normal vaginal bacterial flora of mares. The relation of abortion to sterility according to the French records evidently has little significance, because of the fact, that the incidence of sterility is no higher in mares that have aborted than in those that have never aborted. It is rather striking to note that the proportion of cases of sterility, in which bacteria from the uterine secretion have not been isolated, is very high. Evidence tends to show that the bacterial infection has only a minor significance in equine sterility.

#### (2) BOVINE STERILITY.

Sterility in bulls.—The relative importance of the male as a factor in sterility is negligible, and the average of sterile bulls is only about 1 in 150.

Apart from a small proportion due to other causes, such as congenital deformities and acquired defects, tuberculosis of testicles and epididymis, and Br. abortus orchitis are also responsible for about 50 per cent. cases of sterility in bulls.

Sterility in cows.—On account of its prevalence and economic importance, the study of sterility has of recent years attracted more attention, as it affects the cow more than any other kind of domesticated animals. Infectious sterility in cows is caused by tuberculosis, contagious abortion, contagious granular raginitis and non-specific infection, and the diseases produced by these infections are cervicitis, metritis, salpingitis, cystic-degeneration of ovaries and retained Corpus luteum.

The affection of organs of barren cows varies greatly in different countries according to the circumstances under which the animals are kept. The incidence of uterine diseases is very high among cows kept indoors and fed on concentrated food stuffs for high milk production throughout the year. Ovarian trouble is encountered mainly in underfed herds, bred out-of-doors. Cervicitis is common mostly among cows, kept out-of-doors, pasture-fed and where calving is arranged to take place at the commencement of the season of luxuriant vegetation.

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Salpingitis in the cow and the mare.—In the cow, there is a high incidence of tuberculosis in the tubes and also retention cysts in the ovaries forming a secondary obstruction in the genital tract. Various organisms have been isolated from cases of non-tuberculous salpingitis, the former consisting mainly of streptococci and corynebacteria. However, this disease in the mare has been reported rarely.

The relationship between contagious abortion and sterility in the cow.—Records show that contagious abortion first severely affects a herd of cattle, abortion takes place during early pregnancy and the incidence of sterility is evidently low. Subsequently when the disease has run its course for some time, abortions are fewer and occur later in pregnancy, but accompanied by a large incidence of premature births, retained placenta, severe endometritis and sterility. Fertility is much decreased in cows that proved to harbour B. abortus infection. The effect of the Br. abortus infection therefore, seems to aggravate some other concurrent infection which was prevailing in the herd and transmitted venereally by bulls.

#### The Pathogenesis of Bovine Endometritis,

This condition occurs either following upon B. abortus infection or independently. The following organisms are mainly responsible:—

- (1) B. abortus infection causing merely superficial alterations.
- (2) Pure streptococcus infection; or with other bacteria.
- (3) Pure B. pyogenes infection; or in combination with other bacteria.

After final healing the uterus is often totally unfit for conception.

Venereally transmitted Infections in the Bovine and Equine Species.—The most common infections transmitted and associated with sterility have been described as Coital exanthema; Trichomonad infection, and Streptococcic infection in bovines and Friedlander infection (a bacterial infection with Encapsulatus (Klebseila) genitalium which could be mechanically transmitted by stallion during coitus, with subsequent cervicitis, metritis and sterility) in equines. [B. S.]

## **REVIEW**

Index Veterinarius, 1933, Vol. 1, No. 1, April 1933, Issued quarterly by the Imperial Bureau of Animal Health, Weybridge, 304 pages, Annual subscription £ 4.

Recent years have seen a tremendous increase in the output of periodical scientific literature, which is diffused in the compass of a large number of journals in the diverse languages of the world. It has been increasingly felt, therefore, that unless a sort of bird's eye-view of the world's publications could be made available at one place, workers could not hope to keep abreast of the rapid strides that were being recorded in the realms of science. Moreover, it is neither practicable for every library to subscribe to the whole medley of journals, nor for an individual worker to be able to read every language or see all articles. The enterprising Imperial Bureau of Animal Health at Weybridge has been endeavouring to supply this real need amongst English-speaking research workers by the publication of abstracts of the more important articles in the form of a monthly Veterinary Bulletin, which has already created a place for itself in Veterinary literature and has enlisted a large amount of support. The Bureau has now decided to extend its helpfulness by publishing a very comprehensive index of articles published, bearing upon all aspects of Veterinary Science and the first issue of Index Veterinarius marked April 1933, is now before us for review.

This issue covers the indexing performed at the Bureau Head Quarters during the first quarter of 1933, and it is announced that subsequent issues will similarly deal with the indexing carried out during the corresponding previous quarter. The system of classification adopted is satisfactory, and the required information can be obtained with the minimum of trouble. This quarterly issue forms a complete alphabetical index of the subjects dealt with, as also of authors' names along with all the relevant details (year, full title of article, volume, page, and translation of subject-titles where the original medium of the article is one of the less familiar foreign languages). Conditions due to specific organisms or parasites have been indexed under the name of the causative agent, and a heading consisting of several aspects of a problem has been suitably sub-divided under general, control, diagnostic tests, treatment, bovine, porcine, etc. The nomenclature of the American Society of Bacteriologists has been generally adopted. The articles have been fully cross-indexed under headings of subject, name of the country of incidence, species of the animal and names of the organs affected. The list of journals, which it is proposed to cover in the index, is comprehensive and enumerated on pages 5 to 36. Since it has been necessary to produce it on a

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duplicator, the Index has certain short-comings, for instance, the issue is of necessity rather bulky, but it is clearly printed and bound with a semi-stiff cover similar to that of the Veterinary Bulletin. It is to be hoped that it may yet be possible, following adequate response from supporters, to issue this quarterly index on the printing press, which, it is believed, will eventually assist in the reduction of the cost of production, and hence in enhancing its popularity. The question whether the index could be suitably combined with the Veterinary Bulletin, either as a supplement or with every third monthly issue of the latter deserves consideration, as it would possibly result in a larger and more helpful support for the newly instituted index under the auspices of its more well-established predecessor. The Index Veterinarius is welcomed as a very substantial help to Veterinary workers, and the Bureau is to be warmly congratulated on this new enterprise. [S.C.A.D.]



#### A VETERINARY ENTOMOLOGY FOR INDIA

BY

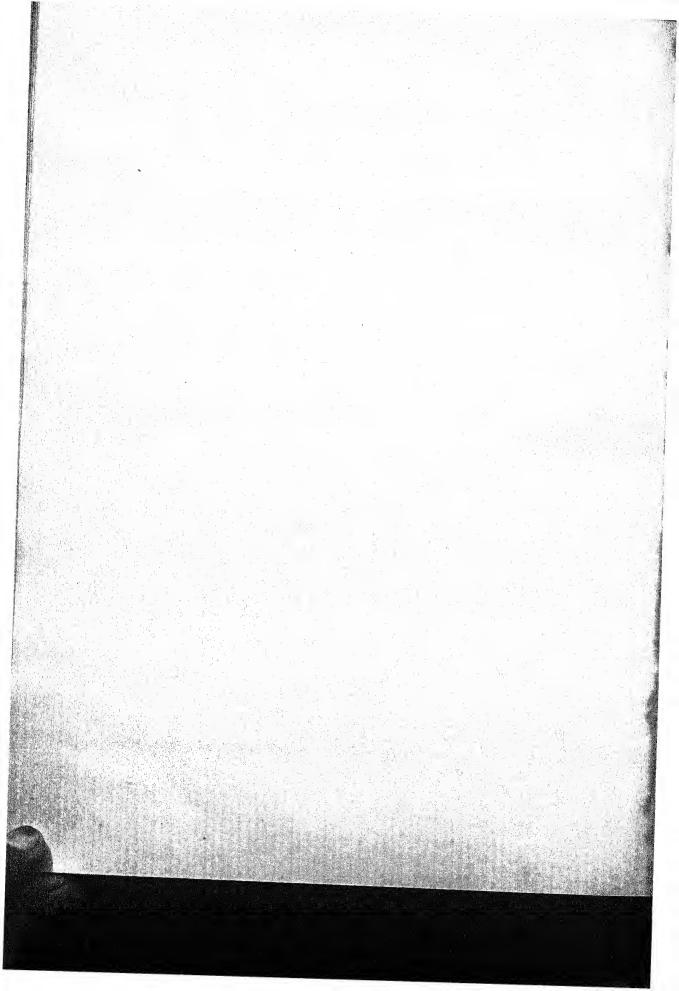
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ANI

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The first of the series of articles entitled as above appeared in 1927 in Vol. I, part I, of the Journal of the Central Bureau for Animal Husbandry and Dairying in India and the last or part XIV in Vol. I, Part III, of the present Journal. It is understood from one of the authors of this series that there are yet several more parts awaiting publication and that if they appear at the present rate of quarterly instalments, it is not likely to require less than 3 to 4 years for the entire series to be completed. The disadvantages of such a protracted method of publication are obvious, for the Veterinary workers for whom these articles are primarily intended are liable to lose touch with the earlier parts of the series by the time the later parts have made their appearance. Moreover, there would appear to be at the present time a genuine need for a general text-book on veterinary entomology which might be used for ready reference by workers in this country. It has therefore been decided to discontinue publication of further articles of the series and the authors have undertaken to complete the work, as early as possible, in the form of a text-book for publication as a Scientific Monograph of the Imperial Council of Agricultural Research.





## APPENDIX

## INSTRUCTIONS TO AUTHORS OF PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH.\*

- 1. All manuscripts should be clean, clear and carefully revised. Only one side of the paper should be used, and as far as practicable the original type-written copy and not a carbon copy should be sent. Capitals should be sparingly used, and all the necessary punctuation should be done in the MS. and not left for introduction in proofs.
  - 2. The title of a paper should not be lengthy.
- 3. It is desirable that the MS, should have suitable heads and sub-heads. In numbering the principal divisions of a paper roman numerals should be used. The use of arabic figures and (a), (b), (c), etc., is generally reserved for numbering the sub-divisions coming under each head.
- 4. Articles submitted for publication either in the Indian Journal of Agricultural Science or in the Indian Journal of Veterinary Science and Animal Husbandry should be accompanied by abstracts for publication in Agriculture and Live-stock in India. Abstracts should be concise, but should be long enough to explain the matter dealt with; ordinarily no abstract should exceed 200 words.
- 5. When a word or line is intended to be printed in *italies* it should be underlined with a single line, in sm. cap. with two lines, in CAPITALS with three lines, and when in **antique** (heavy type) with a wavy line (\_\_\_\_\_\_).
- 6. In decriptive matter, numbers under 100 and all numbers occurring at the beginning of a sentence should be in words.
- 7. Local names for crops, technical operations, etc., should be defined where they first occur in the text, e.g., rabi (spring crop). The use of local weights and measures should be avoided as far as possible. Vernacular names, such as jowar, bajri, should be in italics without a capital letter, and each such name where it first occurs should be followed by its scientific equivalent in brackets, e.g., jowar (Andropogon Sorghum). It is usual to write the initial letters of varietal names in capitals, e.g., Striped Mauritius, Dharwar-American cotton and Broach cotton.
- 8. Botanical and zoological names are printed in italics and should be underlined in the MS., e.g., Triticum vulgare L.; Diplodia Corchori Syd.; Pyrilla aberrans

<sup>\*</sup> Spare copies of these Instructions can be had on application to the Secretary, Imperial Council of Agricultural Research (Publication Section), New Delhi.

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Kirby. The International Rules of Botanical Nomenclature and the International Rules of Zoological Nomenclature should be followed. The names of chemical substances should not be written with a capital letter; they are printed in roman type (e.g., calcium carbonate, prussic acid).

- 9. The following and similar abbreviations may be used freely:—viz., e.g., i.e., mm. (millimetre), cm. (centimetre), grm. (gramme), mg. (milligramme), c.c. (cubic centimetre), sp. gr. (specific gravity), lb. (pound), cwt. (hundredweight), in. (inch), ft. (foot), oz. (ounce), md. (maund), sr. (seer), ch. (chattak). Other abbreviations should be used sparingly, if at all.
- 10. References to plates should be given within brackets, without prefixing the word "see" or "cf.", in the MS. itself, and should not be left over for introduction in proofs. For example, "The parasite (Pl. X, fig. 4) was present late in 1906".
- 11. The word "Table" is preferable to "Statement", and tables should be numbered consecutively in roman figures. Each table should have an explanation as a sub-head. It is more convenient for reference if tables can be printed horizontally; for this purpose they should not exceed in width the printing measure of the page (5"). Example—

Table IV.

Results of water-saving experiments on wheat (Pusa 12) at Gungapur, Haripur and Sargodha, 1916-17.

	No. of irrigations in cluding the preliminary watering	YIELD PER ACRE IN MAUNDS AND SHERS		AVERAGE YIELD PER ACRE	
Station		Grain	Straw	Grain	Straw
Gungapur Haripur Sargodha	One	Mds, Srs.  12 19½ 8 31 8 12½	Mds. Srs.  20 10  19 14  25 27½	Mds. Srs.	Mds. Srs.

12. References to literature, arranged alphabetically according to authors' names, should be placed at the end of the article, the various references to each author being arranged chronologically. Each reference should contain the name of the author

(with initials), the year of publication, the abbreviated title of the publication, volume and page. In the text the reference should be indicated by the author's name followed by the year of publication enclosed in brackets; when the author's name occurs in the text, the year of publication only need be given in brackets. If reference is made to several articles published by one author in a single year, these should be numbered in sequence and the number quoted after the year both in the text and in the collected references. This system of referencing is the same as is used in the Biochemical Journal with slight modification and will be clear from the following illustration:—

The work of Osborne and Mendel (1919, 1, 2) and Steenbock and Boutwell [1919] had indicated an association of the fat-soluble vitamin with the green parts of plants. This view was examined by Coward and Drummond [1921] who reported that vitamin A was not synthesised by etiolated shoots but that green leaves were active in its formation. Another worker [Wilson, 1922], on the other hand, found that etiolated shoots if given in sufficient quantity could supply the fat-soluble vitamin and that this factor was therefore formed in the absence of light.

#### REFERENCES.

Coward, K. H. and Drummond, J. C. (1921). Biochem. J. 15, 530. Osborne, T. B. and Mendel, L. B. (1919, 1). J. Biol. Chem. 37, 187.

(1919, 2). J. Biol. Chem. 41, 549. Steenbook, H. and Boutwell, R. (1919). J. Biol. Chem. 41, 149. Wilson, J. (1922). J. Biol. Chem. 51, 455.

Abbreviations, as far as possible, should follow the system adopted in "A World List of Scientific Periodicals" published by the Oxford University Press.

13. Papers should be complete when submitted for publication. As alterations and additions at the proof stage cause both additional expense and delay, they should be resorted to as little as possible. In making corrections in proofs the recognized symbols which will be found in the "Standard Dictionary" should be used. Second (page) proofs will be submitted to authors who should return them promptly.

#### Illustrations.

- 14. As the format of the journals has been standardized, the size adopted being crown quarto (about  $7\frac{1}{8}'' \times 9\frac{5}{8}''$  cut) no text-figure, when printed, should exceed  $4\frac{1}{2} \times 5$  inches. Figures for plates should be so planned as to fill a crown quarto plate—the maximum space available for figures being  $5\frac{3}{4} \times 8$  inches exclusive of that for letterpress printing.
- 15. Photos or drawings for illustration should accompany the manuscript and each should bear on the reverse side the name of the paper to which it relates together with the title or legend, figure or plate number, and the size to be repro-

duced. When giving instructions for reduction linear measurements are understood; thus, "half-size" means reduce to half the length and breadth, not half the area. A photograph should not be rolled up, nor pinned, and should always be packed flat. A complete list of plates and figures should always accompany the paper.

16. Line drawings should be made with clear black lines on smooth white paper, preferably Bristol board. Rough paper should be avoided. Care should be taken that all the lines are drawn firmly; scratchy or grey lines, produced by the ink being thinned down, are not permissible. Drawings should be larger than the required size. All lettering should be neatly and clearly put in, care being taken to make all lettering sufficiently large to stand reduction.

17. For half-tone work, copy should be made on glossy silver paper and of the same size or larger than the size required.

18. For three-colour work, copy may be oil-painting, water-colour, coloured photograph or coloured transparency, and larger than the size required. In preparing copy, one should use only the primary colours, in any combination, as only inks of primary colours are used in printing. Originals can be enlarged, if necessary, but this should be avoided if possible.

19. For detailed instructions regarding preparation of illustrations, it would be of advantage to refer to Mr. C. M. Hutchinson's article on "Photographic Illustrations" in the Agricultural Journal of India, Vol. XI, Pt. 3, July 1916, and Mr. A. W. Slater's paper on "The Preparation and Reproduction of Scientific Illustrations" in the Proceedings of the Third Entomological Meeting, 1919, which has been reprinted as Bulletin No. 114 of the Imperial Institute of Agricultural Research, Pusa.

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<sup>\*</sup> Agriculture and Livestock in India. 'The Indian Journal of Agricultural Science' and 'The Indian Journal of Veterinary Science and Animal Husbandry' replaced the following publications of the Imperial Department of Agriculture in India with offect from January 1931---

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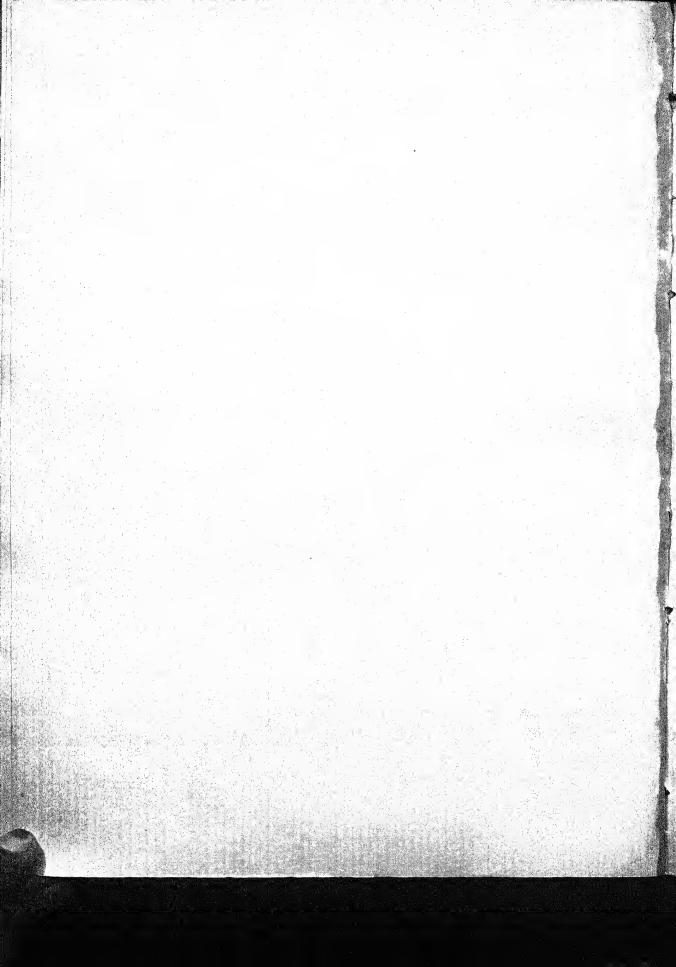
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## ORIGINAL ARTICLES

## INCIDENCE OF EQUINE ENCEPHALO-MYELITIS IN AN INDIAN CAVALRY REGIMENT.\*

I. HISTORY OF THE OUTBREAK.

COMPILED BY

BRIGADIER H. S. MOSLEY, D.S.O.

(Received for publication on the 6th July 1934.)

(With Plate XVII and two text-figures.)

One of the most disturbing factors in veterinary medicine in India, today, is a disease among horses commonly called Paraplegia. *Kumri* or Muttra paralysis.

This nervous disorder has been attributed to several causes, ranging from a vegetable poison as *ratti* seed or a nutritional deficiency to a protein intoxication and again from an obscure injury to a sequela of either an ecto or endo-corpuscular parasitic invasion, as in the last stages of surra or piroplasmosis.

In this instance, the outbreak of paraplegia, extending over a prolonged period, gave the pathologist and clinician a favourable opportunity to make future investigations and they have given evidence to show that, in this particular instance, they were dealing with a disease analogous to one prevalent in Europe and America, and known as equine encephalo-myelitis.

Before narrating the history of the outbreak, it might interest the readers if a brief account were given of the actual work the regiment was doing at the time, and how the incidence of this disease affected the fulfilment of a military programme.

It had been arranged that during the winter of 1933-34, the Loralai Cavalry Regiment and the Jullundur Cavalry Regiment would exchange garrisons.

This move, which was to be carried out by road, necessitated a march of 45 days and 540 miles. The "halfway house" was about Khanewal, but it was decided that the regiments should meet on the 15th November at Dera Ghazi Khan, 176 miles east of Loralai where transport would be exchanged.

Government mule transport was to be used between Loralai and Dera Ghazi Khan and hired bullock transport between Dera Ghazi Khan and Okara.

The march between Dera Ghazi Khan and Okara is 196 miles and occupies 16 days.

In the Khanewal, Dera Ghazi Khan and Rakhni area, Multan hay was to be issued to the units and west of Rakhni, bhoosa supplied by local contractors.

<sup>\*</sup> Published with the permission of the Editor, Royal Army Veterinary Corps Journal, for which this article was originally intended.

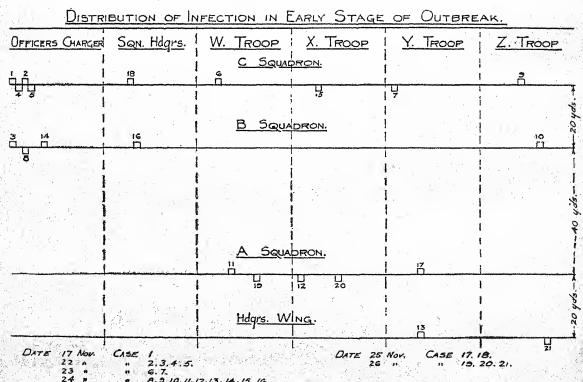
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The Jullundur regiment had been stationed there for 5 years. It left on the 16th October, arrived at Okara on the 30th October and Dera Ghazi Khan on the 15th November (where it halted for 24 hours), transport units were exchanged and the march resumed on the 17th. This regiment in accordance with programme, arrived in Loralai on the 29th November. No case of encephalo-myelitis occurred in the regiment in Jullundur, on the line of march or since its arrival in Loralai.

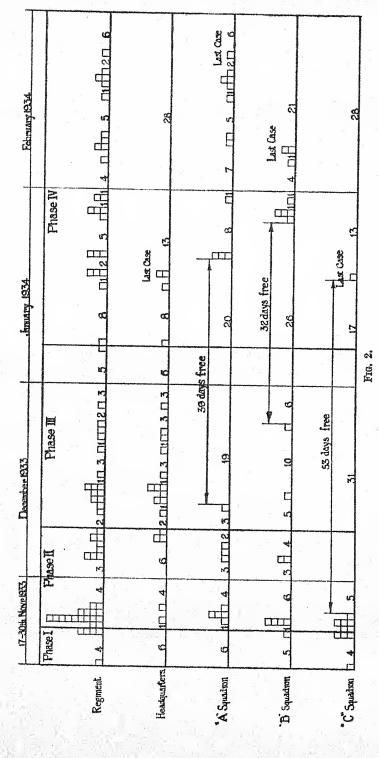
The Loralai regiment had been there for 3 years, and no case of encephalomyelitis had been recorded in the regiment or station.

The regiment left Loralai on the 3rd November and arrived at Dera Ghazi Khan on the 15th.

On arrival in camp, Major "A's" charger of C Squadron was not feeding that evening; next day, the horse appeared well and marched with the led horses; on the 17th November, to Kureshi Camp, a distance of 17 miles. That evening the horse had a rise in temperature and next morning was partially paralysed. This was the first case (Case I).



Frg. 1.



Figures between squares denote number of days. Squares denote number of cases in one day.

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For convenience, the history of the outbreak will be divided into 4 phases.

Phase I. The march between Dera Ghazi Khan and Khanewal (17th—23rd November 1933).

The regiment arrived at Kadarpur Ran on the 21st. Next morning, a charger (Case 2) belonging to Captain "B" of C Squardron, which had been in contact with the first case, developed the disease. That same day, on arrival in camp at Kabirwala, there were 3 more cases—a charger and private pony of Major "A" and a charger belonging to Captain "C" of "B" Squadron. (Cases 3, 4 and 5.)

Uptodate, all affected animals belonged to officers, 4 occurred in C Squadron, and 4 had marched with led horses.

All cases proved fatal except the private pony.

The Officer Commanding of the regiment reports that at Kadarpur Ran on the 22nd November, he noticed that one of the transport bullocks was lying down in a dying condition.

Phase II. Incidence of the disease in Khanewal (23rd November to 8th December).

The regiment reached Khanewal on the 23rd November where 2 troop horses of "C" Squadron (cases 6 and 7) became affected.

The march was temporarily abandoned and an application made for the services of a veterinary officer. Major Heane arrived that evening and remained with the regiment until the 17th April 1934.

Early on the morning of the 24th, as headquarters wing and "A" Squadron had not had a case, they were transferred to a new camp  $1\frac{1}{2}$  miles away and on the far side of the city.

"A" Squadron was picketted on the grass verge of an orchard and headquarters wing 100 yards away, the 2 units were separated by a canal.

The 24th November was the worst day during the outbreak as 9 horses developed the disease (cases 8-16) 2 of which were chargers of "B" Squadron.

The D. A. D. V. S., Lahore District, visited the regiment on this day and, very rightly, considered that the situation required the services of a pathologist. He applied to the Principal of the Punjab Veterinary College who placed Professor Shirlaw's service at the disposal of the army. The Principal also provided veterinary assistants for field work during the investigation.

Oh the 25th November, cases 17 and 18 occurred and on the 26th cases 19, 20 and 21.

On the 26th November, as all squadrons were now affected, it was considered advisable for administrative purposes to transfer "B" and "C" squadrons to the camping area of headquarters wing and "A" squadron.

In this area, there was a 20 acre orchard of young trees, which, it is understood, is ordinarily used for durbars and fairs. The two squadrons were picketted in this orchard.

On the 27th November, 2 horses in "A" Squadron developed fever; the next day 1 in "A" squadron and 2 in "C". This was the beginning of an outbreak of equine piroplasmosis. In 10 days, there were 34 cases. Later, after the regiment was transferred to Multan, there were 3 more cases. 4 of the 37 cases developed symptoms of encephalo-myelitis.

On the 1st December, in consideration of the outbreak of piroplasmosis, it was thought advisable to move the regiment back to its original camp. On the 4th December, piroplasmosis died down but encephalo-myelitis re-started. During the time the regiment was in Khanewal, there were 25 cases of encephalo-myelitis and 6 deaths.

On the 6th December, the march was definitely postponed and on the 8th, the regiment was entrained to Multan.

Phase III. Incidence of the disease in Multan, up to the time of squadron isolation being adopted (8th December 1933 to 7th January 1934).

During this phase, headquarters wing suffered more heavily than others; there were 13 cases in this unit and only 1 case in "A" and 2 in "B"; the total being 16 cases and 5 deaths.

"A" squadron was picketted about 200 yards away from the rest of the regiment; the horses were watered at the same well, although using different troughs. It would not be right to say that "A" squadron was under strict segregation.

Phase IV. Adoption of the squadron isolation (7th January to end of outbreak). This policy was adopted on the 7th January and remained in force until each individual squadron was entrained to Jullundur.

Headquarters wing was left in its original standings. "B" squadron occupied "A" squadron lines. "A" squadron and "C" were transferred to a fresh camp. In this way, each squadron was 500 yards apart and had its own watering arrangements. Cases continued to occur in headquarters wing on the 7th, 16th and 18th (2 cases) January—4 cases in all. After the 18th January, there were no further cases.

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- In "A" squadron, no cases occurred until the 21st January when there were 3 cases. This squadron had been free for 38 days. There was a case on the 30th January and one each on the 8th, 9th, 15th, 17th, 19th and 22nd February and 2 on the 18th February, a total number of 12-cases. The last case was on the 22nd February.
- "B" squadron.—After an interval of 32 days, a case occurred on the 27th January, 3 on the 28th, 1 on the 30th, 1 on the 5th and 2 on the 7th February—total 8 cases. The last case was on the 7th February.
- "C" squadron.—A case occurred after an interval of 53 days, on the 18th January—which was the last case.

During this phase, there were 25 cases and 6 deaths.

Information being received that cases had occurred in Germany after an interval of 80 days, the squadrons were kept in segregation and under observation for 3 months after the last case.

The regiment was finally entrained by squadrons to Jullundur.

Total No. of cases 71, deaths 21 (mortality 29.58 per cent.).

### Remarks-

- (1) All horses had been in the affected regiment 6 months previous to the march.
- (2) Although the 2 regiments marched about the same time, over the same area, used the same camping grounds, obtained fodder and water from the same source, shared the same transport, yet cases only occurred in the Loralai regiment which looks as if the source of infection was confined to this unit.
  - (3) No cases occurred in the mule transport.

## II. CLINICAL OBSERVATIONS, TREATMENT AND EXPERIMENTS IN THE FIELD, ETC.

BY

## MAJOR C. W. HEANE, R.A.V.C.

## CLINICAL OBSERVATIONS.

At the outset it should be noted that the clinical picture presented and the degree of severity of attack in this outbreak was peculiarly varied, and ranged from cases which fell down in the lines completely paralysed, convulsively pawing the air and banging their heads against the ground and causing severe bruising over the

eye, to those which showed only the slightest inco-ordination of movement in the hind quarters.

In the large majority of cases, the first indication of any thing wrong was shown by a marked dulness and depression, and in all except the mildest cases, off feed, and accompanied by a stiffness in gait of the hind limbs.

Temperature was seldom elevated on admission.

When the disease was definitely established, the inco-ordination and loss of muscular power in the hind limbs became very apparent.

The hind toes were dragged at the walk and the tail nearly always elevated.

Inco-ordination was most noticeable when turning round, the animal appearing disinclined to turn on his hind quarters and when doing so there was a marked sinking of the loins. This was the last of the symptoms, to disappear in the process of recovery. Varying directly with the severity of the attack partial or complete sensory paralysis in the hind limbs was shewn, seldom or never extending sufficiently far forward so as to cause complete loss of sensation and control of the fore limbs.

In some cases partial paralysis of the fore limbs only was apparent—the horse crossing his legs at a walk and displaying exaggerated knee action and in all cases of fore or hind paralysis, during movement, the animal appeared to be feeling for the ground.

Hyperaesthesia was pronounced in the early stages of an attack and then appeared to pass off in one or two days. Dilation of the pupil and twitchings of the facial muscles were sometimes observed.

More constant symptoms were:—Pulse, sluggish and feeble; incontinence, and severe cases, complete retention of urine. Faeces were also found to be retained, but without constipation. The heart beat was invariably feebler than normal, but otherwise regular.

In geldings the retractor muscles being involved in the paralysis, the penis would hang down out of the sheath. The lower lip was often paralysed and pendulous.

Urine at the commencement of an attack would be clear in appearance but of a treacly consistence, in severe cases sometimes becoming later muco-purulent and offensive in odour.

Two horses showed symptoms of hemiplegia, the neck turned round to one side and able only to move round in a circle; one of which died and one recovered.

Other symptoms sometimes observed were swelling of both hind fetlocks and paralysis of the jaws.

One case only developed cranial symptoms which appeared in the nature of "fits", with twitchings and contractions of the muscles of the neck, the head held down, and finally falling to the ground, unconscious and struggling. After 10-15 minutes, the horse would get up again and appear quite normal. This animal eventually recovered.

The large majority of fresh cases, as they were discovered, showed no apparent relationship to the previous ones by having been in contact with them in any way or at any time, and cases of actual incontacts developing the disease were rare.

In the last stages of the disease twitchings of the abdominal or pectoral muscles generally supervened. Every cases which collapsed totally in slings unable to stand even with their support proved fatal.

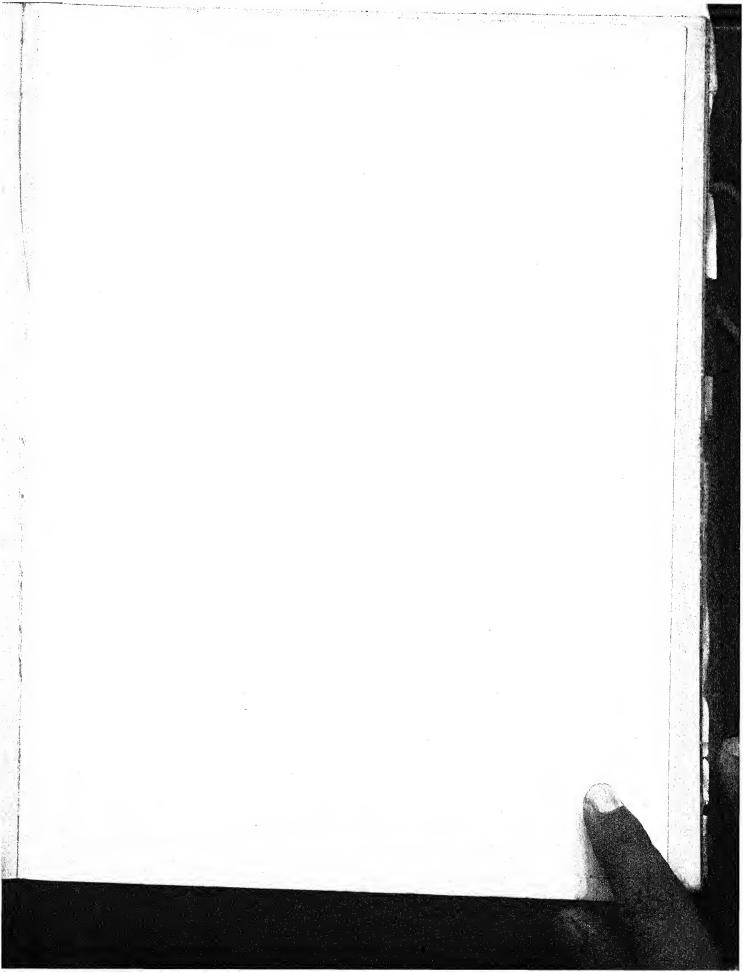
## PROGNOSIS AND MORTALITY.

At the commencement of the outbreak from 17th to 24th November, before comprehensive routine treatment, slinging, etc., could be put in to practice, the mortality worked out at 80 per cent.

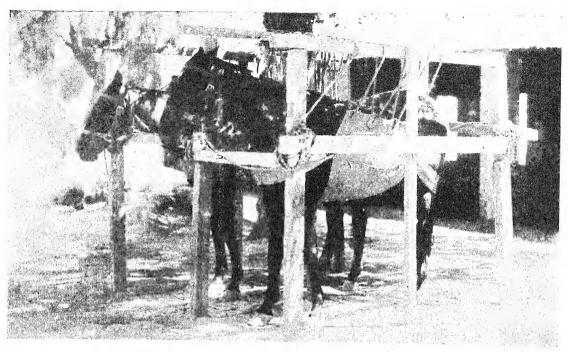
From that time until the end of the outbreak in the following February, 26 per cent. of cases only were fatal. As a general rule those showing only slight symptoms when first noticed were fairly certain to recover under treatment.

In the case of those showing severe symptoms at the outset, which in this outbreak may safely be estimated at about two-thirds, prognosis is difficult.

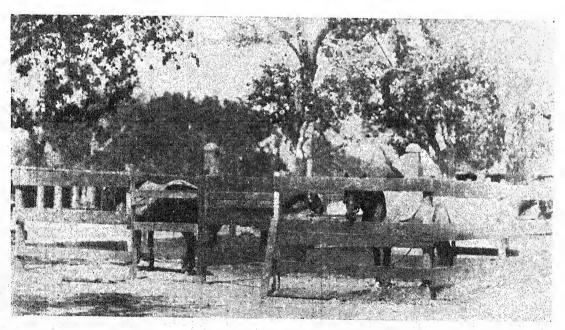
From what I have seen the critical stage of the attack appears to be somewhere about 24—36 hours after the commencement of symptoms. It was found as a general rule that if the animal could be kept on his feet up to 36 hours by means of slings, in practically every case recovery followed. Once the animal had to be left out and was down, however the condition rapidly became worse; but so long as the animal could stand on his fore legs and had the slings to support his hind legs and to preserve his balance, after 36 hours his chances of recovery stood high,



## PLATE XVII.



1. Patients in temporary slings.



2. Temporary loose boxes.

#### TREATMENT.

In all but very mild cases slinging was resorted to immediately on discovery.

The type of sling constructed was as shown in Plate XVII. For "field" use it proved 100 per cent. effective and while able to support the full weight of the animal's body, it had the additional advantage over the orthodox hook and pulley sling that balance was much easier maintained by having complete support on either side as well as in front and behind.

The frame-work consisted merely of stout wooden posts about 6 feet 6 inches high, the only detachable parts being a short post at the breast and one at the croup which were roped on to the uprights.

The sling itself was made from good strong sacking. Four holes were made at each end through which the rope was threaded and the holes stitched round with stout leather to prevent tearing of the material. A rope on each side was then passed through the rear hole and knotted the free end passing over the top bar and down again through the next hole and so on, so that the sling could be tightened or loosened after the manner of a boot lace. Breast plate and breeching, also of sacking, kept the sling in position and were fastened to the sling itself by means of buckles. The standing was bricked and with the downward slope forwards in order to take as much weight as possible off the hind part of the body.

Routine method of treatment which was carried out in all cases on admission and subsequently as a daily measure as far as was considered necessary:—

The animal was first placed in slings. In the early part of the outbreak blood smears were taken and examined, all with negative results.

Twelve ounces of mag. sulph. was administered by stomach tube. The rectum was emptied and an enema used. The catheter was passed in all cases, male and female, and the bladder invariably found full.

Towards the end of the outbreak the effect of hexamine (urotropin) was being tried for its curative properties. Actually only the last six cases received this drug but all were severely affected and all recovered. The dose given was 25 grammes daily in 2 ounces of water, intravenously over a period of 4 days.

Previous to this drug being tried cases were being given subcutaneously, serum taken from the blood of convalescent and recovered cases in doses of 200 c.c. on admission and subsequently 100 c.c. daily. In the more severely affected cases this did not appear to have any appreciable benefit but in all others recovery was definitely more rapid than otherwise was the case. In order not to confuse the issue the administration was abandoned for the time being for the purpose of trying out the effect of hexamine.

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From time to time the following agents were used in the treatment of this disease without any apparent benefit whatever:—

Strychnine, adrenalin, morphine, arecoline, calcium lactate, 2 drams intravenously, naganol, 4 grams intravenously, normal saline intravenously.

All cases if they were able to walk at all, even with the assistance of 4 or 5 men to support the animal, were given forced exercise daily. The effect was very pronounced—after a few paces only the improvement in gait was apparent and this form of treatment I believe to be of real and lasting benefit.

For this purpose also two blocks of four loose boxes were constructed from posts and planks only (Plate XVII) in order to allow convalescent cases to exercise themselves.

Several horses that fell down in their own lines had to be brought to the sick lines with the aid of two bamboo poles, one pushed under the chest and one under the abdomen and a party of men on each side were able to raise the animal on to his feet and with the poles still under him, led forward slowly to the sick lines.

When the case was considered hopeless, destruction was carried out by means of chloroform to admit of the brain being removed intact.

The process of recovery took anything from 3 weeks to 2 months.

### POST-MORTEM CHANGES.

Naked eye changes were very few but fairly constant.

There was always evidence of a mild gastro-enteritis occurring in patches.

There was always present cystitis of varying intensity with thickening of the bladder wall. The spleen was found sometimes to be considerably enlarged. Vessels of the cortex of the brain invariably showed some congestion.

EXPERIMENTS AND CENERAL OBSERVATION IN THE FIELD.

The disease appears to attack all classes of the horse irrespective of breed, age or sex.

During the period in which the disease was confined to Headquarters Squadron, on the recommendation of Major G. A. Kelly, R.A.V.C., D.A.D.V.S., Lahore District, it was decided to test the properties of serum taken from convalescent and recovered cases, in a prophylactic and curative sense.

The serum was prepared as follows:—About one litre of blood was drawn off from the jugular vein into a sterile flask and the blood allowed to clot and the serum to be expressed in the normal way, each litre of blood yielding about 350-400 c.c. of serum. After 24 hours the serum was drawn off and to every 100 c.c. was added 10 c.c. of a 5 per cent. solution of phenol.

As an experiment, half the number of the horses of Headquarters Squadron, approximately 50 were given 100 c.c. of this serum in an attempt to confer on them passive immunity. The result unfortunately could not be considered in any way conclusive as from the time these inoculations were carried out no further cases occurred throughout the whole squadron, with the exception of one which had received serum 48 hours previously and which was undoubtedly well advanced in the incubative stage.

At one period the possibility of an excess of bara grass (Sorghum Halepease) in the hay, said to have a cyanogenetic action, being in some way responsible for the disease, was considered and it was decided as a test to feed this grass only to one selected horse.

The Grass Farm at Multan collected about 60 lbs. of baru for this purpose. However, the effect it had on the animal appeared to be nil.

Food deficiency as a contributory factor in the cause of the disease was also thought to be a possibility.

By the orders of the D. D. V. S., Northern Command, a supply of mineral feeding flour and pot iodide was arranged for and was given daily to all horses in Headquarter Squadron, 2 ounces of the flour daily being mixel in each midday feed. This was commenced on the 25th of January, but subsequent to it being put into operation there were no further cases in this squadron—the last case having occurred on the 18th January—and in consequence this experiment was not proceeded with further.

The period of incubation and method of natural infection is not definitely known but the fact that on one occasion after "A" squadron had been clear of the disease for nearly six weeks and 14 days in isolation 3 fresh cases appeared in the squadron at precisely the same time, not incontact with one another in any way, seems to indicate that the period of incubation, whatever it may be, is constant, and to the possibility that it is spread by some common transmitting agent such as biting fly or mosquito. The way in which fresh cases appeared usually having no apparent relationship with any previous ones would also tend to support this supposition of the means of spread.

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Two attempts were made in the lines to pass on the disease from one animal to another.

(1) By removal of spinal fluid taken from a horse in the acute stage of the attack and inoculating into the spinal canal of a healthy animal.

About 2 c.c. of spinal fluid was removed by means of an intrathecal needle, from a case in slings at the time. An experimental horse was then thrown by means of side lines and the 2 c.c. of spinal fluid injected intrathecally. This failed to set up the disease.

(2) Another experimental horse was placed alongside a recent acute case in slings and watered from the same bucket. Saliva was also transferred at intervals to the healthy horse's mouth and grass given contaminated by urine from the affected case. This also failed to communicate the disease.

In two cases, acting on the supposition that possibly an increased production of cerebro-spinal fluid might account for the disturbance of co-ordination I removed with an intrathecal needle 50-60 c.c. of spinal fluid in each case, but without effect.

On the 2nd February systematic taking of evening temperatures of all horses in the Regiment was initiated on the recommendation of the D. V. S. in India. As a result an interesting feature in the nature of the disease came to light.

It revealed that in a certain percentage of cases and possibly in all, the symptoms of paresis are preceded by preliminary phase of pyrexia lasting from 2 to 4 days followed by a return to normal 1 to 2 days before the onset of actual symptoms of paralysis.

Soon after temperature taking commenced, cases of fever occurred at irregular intervals amongst the horses of "A" squadron only. Altogether 10 horses showed temperatures varying from 102°-106° F. As each case of fever was discovered it was isolated. Of these 10 cases, 6 subsequently developed encephalo-myelitis in the manner described. It would seem probable that the other 4 were infected but not sufficiently severely to set up clinical disturbance.

These were the last cases to occur in the Regiment.

The fever was not accompanied by any systemic disturbance whatever and the only abnormality noticed was some injection of the conjunctival mucous membranes and in all cases which developed the disease, swelling of both hind fetlocks.

Blood smears from all these cases were examined daily with negative results.

Had cases continued and this preliminary fever found to be a constant factor, the method of control would have been very much simplified. Again, had further cases occurred it was intended for the purpose of definitely determining whether or not serum had any protective value, to regard any rise of temperature as a premonitory symptom of the disease and give each one 200 c.c. of serum either subcutaneously or intravenously.

It is just possible that the virus may be present in the blood stream only during this febrile period and thus making the animal infective to others only at this time, which might account for the difficulty of infecting a healthy animal from a diseased one by experimental methods.

It was unfortunate from the investigation view point, that the outbreak ceased just at a time when the clinical study of the disease had reached an interesting stage.

Local conditions of climate and humidity may have some influence on the activity of the virus and cold and dampness would appear to enhance its virulence. More cases occurred during the real cold weather and when there was dew than at other times. The very large majority of cases were discovered in the early mornings. The disappearance of the disease coincided with the beginning of warmer weather.

The question of whether or not immunity is developed is interesting. It is not determined but the fact that all cases which did not end fatally to all appearances recovered completely and that none have shown any sign of relapse seems to lend weight to the supposition that immunity is set up and that in consequence the serum has some clinical value.

The possibility of recovered cases being "carriers" cannot be ignored.

## III. PATHOLOGICAL INVESTIGATION OF DISEASE IN HORSES OF THE 13TH LANCERS AT KHANEWAL AND MULTAN.

BY

## J. F. SHIRLAW, M.R.C.V.S.,

Professor of Pathology and Bacteriology, Punjab Veterinary College, Lahore.

The report deals with the complete investigation into the outbreak of paraplegia affecting the horses of the 13th Lancers. Mention is made of the initial steps which have been taken to make an attempt to arrive at a diagnosis, and the

writer is in a position to record some very accurate information concerning the essential nature of the disease and an account of the various steps that have been taken to establish a diagnosis.

Throughout the course of this outbreak, the writer has regularly received such pathological tissues as he considered necessary, and a detailed post-mortem account of every horse that either died or was destroyed in extremis.

It was decided, as an initial step, to obtain the most accurate information possible concerning the post-mortem appearances, and secondly, the essential tissue changes occurring throughout the organs.

In all, 14 horses were subjected to a post-mortem examination; the numbers of these horses are H. 156, H. 159, H. 332, H. 282, H. 149, H. 42, H. 513, H. 88, H. 103, H. 515, H. 288, H. 416, H. 286, H. 312. It can be definitely stated that the macroscopic appearances were practically identical in every case. The most striking feature of the post-mortem examination is the relative absence of any gross pathological picture. In one or two cases the pathological findings might, to a casual observer, be termed negative. In the majority of cases, a mild catarrhal inflammation of the gastro-intestinal tract was noticed. The parenchymalous organs appeared to contain more blood than normal without any evidence of an inflammatory process. The spleen seemed slightly softer in consistence but was not distended. The kidneys appeared superficially normal, but on mesial section streaks of hamorrhage radiating from the medulla were found. The bladder contained, in most cases, urine of an altered character. In some cases this was thick and syrupy in appearance and contained much sediment; in other cases the urine was full of blood. The bladder wall, in nearly every case, showed more or less extensive hæmorrhage in the mucous coat. In one case a severe hæmorrhagic cystitis was present. The presence of a straw coloured fluid in the peritoneal and the pericardial sacs was described in a few cases. This fluid was free of cells, contained no bacteria, was poor in fibrin and appeared more of the nature of a transudate than an exudate. The lymph glands were moderately swollen and in some cases showed hæmorrhages.

The most typical features of the disease were found in the brain and spinal cord. A superficial examination intimated the presence of a meningitis, but on more critical examination it was seen that the lesion consisted of an abnormal fulness of the vessels of the meninges and of the choroid plexus, without the presence of any exudate in the meningi. The cut surface of the brain showed hæmorrhages in the cerebral cortex, especially in the area of the choroid plexus. Punctate hæmorrhages were observed throughout the brain substance and in the brain stem as well as in the cortex. In many cases these hæmorrhages were extensive, and

extended to a depth of several millimetres below the meninges, separating this membrane from the ependyma. In one case, a true meningitis with the presence of an exudate in the meninges was noted. The spinal fluid was in every case clear and was not increased in quantity, and on examination there was no increase in cellular content. The fluid appeared blood-tinged in one or two cases, but this was probably caused by the accidental rupture of capillaries. The presence of bacteria could not be detected in this fluid from any case, either by direct microscopic examination, or by selective cultural methods.

The spinal cord showed either extensive or patchy hamorrhage under the meninges, hamorrhages extending for considerable depth round the nerve roots, and in some cases, a gelatinous exudate under the duramater.

It will be seen then, that the most important lesion consisted of what might be termed a sub-acute inflammation of the brain. spinal cord and coverings with hæmorrhages of varying intensity occurring chiefly under the piamater.

## BACTERIOLOGICAL EXAMINATION.

The following materials from post-mortem cases were examined in every case:—

- (a) Brain
- (b) Spinal cord.
- (c) Spinal fluid.
- (d) Blood.
- (c) Liver.
- (f) Spleen.
- (g) Kidney.
- (h) Bladder.
- (i) Urine.

Tissue films were prepared from all these organs and tissues, and in no case could any bacteria be detected. The exudate which was found in the cord proved to be negative on examination. The culture media used were:—

- (a) Blood agar.
- (b) Coagulated blood serum.
- (c) Marmorek serum medium.

while sterile tubes containing fluids such as blood and cerebro-spinal fluid were also incubated after cultures had been made. In four cases a strepto coccus was obtained, similar in type in all cases. This organism was definitely haemolytic and easily obtainable in pure culture from the brain pulp of the four cases.

Further experiments were carried out with pure broth cultures of this organism to test out its virulence in small laboratory animals and in horses. Rabbits proved very susceptible to inoculation, dying within 48 hours. One horse was selected for massive repeated intravenous inoculations of pure broth culture and showed no effect whatever. The most striking feature of the disease, therefore, is the absence of any bacteria which could be considered as of etiological significance. A similar examination for protozoan parasites was negative.

## TRANSMISSION EXPERIMENTS.

These were commenced simultaneously with the main pathological examinations. One college horse was inoculated with 5 c.c. of blood removed at postmortem from horse No. 156, post-mortemed by the writer on the 28th November and a second horse was inoculated with 5 c.c. of cerebro-spinal fluid from the same case. No reaction followed and it was found impossible to transmit the disease by this method. At this time some considerable suspicion attached to the feed of the affected animals, and it was decided to observe the effect of feeding experimental animals such as rabbits and guinea pigs with the stomach ingesta of animals that had succumbed to the disease. A second part of this experiment consisted in feeding similar animals on bajri (Pennisetum typhoideum) and wheat that had been soaked in the large intestinal contents of diseased animals. This experiment was carried out for a period of fifty-six days. All these animals remained healthy. The possibility of forage poisoning was then considered, and work was undertaken in conjunction with the Chemical Examiner to the Punjab Government, to elucidate this possibility. An examination of the stomach ingesta of diseased animals proved consistently negative for group alkaloid tests. In two cases, arsenic was detected in such specimens. This finding was rather startling, as no source of such poisoning could be traced. It was reported, however, by the Chemical Examiner, that the amount of arsenic present could not be held responsible for any disease symptoms. About this time, as the result of histological examinations of the brain suspicion attached to the possibility of a filterable virus being the etiologic factor, and as the lesions of the disease were chiefly confined to the brain and spinal cord which had proved sterile on bacteriological examination, it was decided to attempt the transmission of the disease to experimental animals with brain material. It became the routine, therefore, to preserve the brain and spinal cord from all post-mortem cases

in 50 per cent. glycerine. These specimens were despatched to the laboratory with all possible speed, and saline suspensions were made from these tissues and inoculated subdurally into rabbits and guinea pigs. Rabbits have proved quite refractory to this experimental inoculation and two guinea pigs inoculated died without symptoms. These experiments indicated that the disease could not be transmitted to experimental animals of this type with the exhibition of nervous symptoms, characteristic of the disease in horses.

One of our college horses was inoculated intrathecally with 2 c.c. cerebro-spinal fluid from an acute case of the disease that had been destroyed in extremis. At the time of inoculation a similar amount of fluid was removed from the experimental case to avoid any intracranial pressure, which might be likely to produce fallacious results. Within 24 hours, this horse showed definite signs of malaise and in 36 hours showed signs of hyperaesthesia, inco-ordination of movement and general symptoms of meningitis. The fluid injected was bacteriologically sterile. There was no elevation of temperature, and the injection had been performed without any apparent injury to the cord or its coverings. The symptoms abated within three days, but a definite inco-ordination was noted for some time after. The interpretation of this experiment is rather difficult. If the symptoms of meningitis had been caused by injury at the time of inoculation, one would have expected to find them of a more intense nature, and of a more protracted duration. A possible explanation is that certain toxins were present in the fluid and that these were responsible for the manifestation of disease symptoms which were strictly comparable with those observed by the writer in naturally occurring

This experiment was repeated upon two horses with entirely negative results.

The most striking and important inoculations performed on experimental horses were those in which brain emulsion prepared from an acute case of the disease that had been destroyed in extremis was used. Three horses were used in this experiment. These horses were army casters in good condition and were sound in health and action. The first horse was trephined and was inoculated into the duramater with a few drops of the saline brain emulsion. The second horse was inoculated subcutaneously in the area of the left temporal plexus and the third horse was inoculated with 1 e.c. of the more fluid portion of the brain emulsion intrathecally. These inoculations were performed on the 11th January, 1934. The horse inoculated intradurally showed definite symptoms of inco-ordination of movement on 30th January, and on the same day the horse inoculated in the temporal region showed a similar inco-ordination of movement of the hind limbs, of a more exaggerated type. The third horse failed to react. This inco-ordination

gradually became progressive and for some little time hyperaesthesia with loss of the ocular reflexes was noticed in the horse that had been inoculated intradurally. The inco-ordination consisted of a loss of power in the extensor group of muscles of the hind limbs. Both animals showed a peculiar dragging movement of the hind quarters, difficulty in turning, crossing of the feet, and, in short all the typical symptoms of the disease which had been noted by the writer at Multan.

These horses were destroyed by chloroform on 5th April 1934 and tissues were removed from them for examination. At the time of destruction the horse inoculated in the temporal region was showing slight inco-ordination, while the horse inoculated intraducally was sound in action. It can be deduced from this important experiment that, by this method of inoculation, it was possible to reproduce the disease in a mild form in the horse. A definite incubation period of 19 days elapsed between the inoculation and the development of symptoms, and the fact that both horses developed symptoms on the same day is of further importance.

## ESSENTIAL PATHOLOGY.

The chief feature of this consists of hamorrhages in the parenchymatous organs with very slight changes in the structure of the organ. In about half the cases examined, these hæmorrhages were unaccompanied by cellular degeneration, and it is probable that apparent degeneration is caused more by post-mortem changes which are always rapid under tropical conditions. A great difficulty in obtaining fresh tissue is caused by the simple fact that the tissue commences to decompose at a rate quicker than the infiltration of the fixative used. These fixatives were of the pieric or bichromate series and are rapidly acting, and yet, in spite of their use, cellular fixation was not sufficiently good definitely to establish the important point whether parenchymatous degeneration does occur. The most important pathological change, one which is expressed throughout all the tissues, is in the blood-vascular system, and it is due to these changes that hæmorrhages occur. The change consists of a sub-acute endarteritis which is slowly progressive. and results in a thickening of the arteriole and a narrowing of its lumen. The process extends through the intima and a coarse thickening of the fibrous coat of arterioles occurs. Thrombosis is occasionally seen. The change affecting arterioles is accompanied by a venous stasis. The veins become so abnormally full of blood that they spontaneously rupture, causing interstitial hamorrhage, which, in highly vascular organs like the spleen, are very extensive. So complete is the break-down of the vascular system of the spleen, that slices of spleen after fixation in 5 per cent. formaline appear like blood clots, being tough, and of a leathery consistense. The hæmorrhages in the meninges may be either slight or

extensive. They are constantly present; and may extend under the piamater, in the choroid plexus, and to a considerable depth, through the sulci of the brain. These hamorrhages are constant throughout the cut surface of the brain, and may be seen in the olfactory lobe, the pons, thalamus and the brain stem as well as in the cerebellum. In the spinal cord, hemorrhages extend around the nerve roots. They may be under the duramater or more commonly under the piamater extending for a considerable distance. The nerve roots may be so densely intiltrated by the hemorrhages that pressure necrosis of the nerve elements may be seen. A study of these lesions convinces one that the nervous symptoms of the disease are partly due to these hæmorrhages occurring within or around important nerve structures.

The most informative fact about the disease is obtained from a microscopical examination of the brain. The capillaries and arterioles of the choroid plexus and its branches in the cerebral cortex are thickened, and in the majority of cases there is a definite perivascular infiltration of mononuclear cells, this infiltration extending into the neuroglia cells. In the minority of cases this infiltration or "cuffing" of the arterioles and capillaries is not so well marked, but in these cases, there is an extensive, diffuse infiltration of the neuroglia. There is a degeneration of the nerve cells in the cerebral cortex and a degeneration of the cells of the cerebellum (cells of Purkinje). The degeneration affects chiefly the nuclei of the cells. The general appearance of the microscopic lesion in the brain tissue is very similar to that described in infections with neurotropic viruses, the only difference being that in the latter disease, hemorrhages are not encountered and intranuclear inclusions known as "Joest-Degen" bodies are found. In this disease such bodies have only been seen doubtfully in one case. It is noteworthy in this connection that most of the cases were acute, whereas in cases of Borna disease the symptoms of the disease are of longer duration. In examination of a few cases a definite meningitis with exudation was present. This lesion was more common in the spinal meninges.

Most interesting lesions were found in a horse which had recovered from an acute and serious type of the disease. This horse, after recovery, appeared perfectly normal, and as a point of interest it was decided to perform a post-mortem upon it. Hæmorrhages were present in the parenchymatous organs. The meninges were grossly thickened and adherent.

Extensive perivascular infiltration was present around the vessels, and throughout the neuroglia; the nerve cells of the cerebral cortex showed degeneration, while persistent hæmorrhages were present in the grey matter of the brain around the choroid plexus.

On such findings, the apparent sound health of the animal and the freedom from nervous complications seemed a striking feature.

The disease appears as a specific meningo-encephalitis of the horse. This disease, or a disease of a similar nature, has been described in many countries: and on a review of the literature pertaining to meningo-encephalitis of the horse, the outbreak which occurred in the horses of the 13th Regiment bears a very striking similarity, to those outbreaks recorded in America, in which country serious epidemics of the disease have occurred. It is only in the last decade that any attempt has been made in America to elucidate the essential pathology of this disease. Previously, it had been considered in some way associated with the feeding of the horses.

In Europe generally, and in Germany especially, a similar disease has been known for many years, for at least a century—so called enzootic cerebro-spinal meningitis or "Borna" disease. This disease is enzootic in areas, tending to assume epizootic characters, and in such infected areas, cyclic incidence occurs at intervals of every few years. The incidence seems controlled to some extent by climatic factors. The disease appears infectious, although one of its most striking peculiarities is that it does not spread in the same way as infectious diseases usually do. During the course of an outbreak, animals may become infected in batches, and, when breaking out on a horse-breeding farm, may occur only on one part of the farm. In certain outbreaks, only one or two animals may sporadically be affected, and the disease may tend spontaneously to disappear without any further spread, while in other outbreaks, the disease may sweep through the horses assuming the proportions of a veritable epidemic with a high rate of mortality. In American outbreaks, mortality averages about 40 per cent.

Very little is known concerning the transmission of the disease under natural conditions. Infection is possibly by way of ingestion, but a very strong suspicion attaches to the nasal passage as an atrium of infection. Infection seems predisposed to by a mild gastro-intestinal catarrh. Drinking water has been found to convey the infection, and small animals such as rats, squirrels, mice, rodents generally, and even birds, are susceptible to the virus of the disease. There is a strong tendency to incriminate them as carriers during the course of an outbreak.

Although it has been a fashion to designate this disease as forage poisoning, all attempts to prove the etiological significance of fodder have failed. A very strong suspicion attaches to the possibility of recovered cases of the disease acting as carriers. The most certain way of infecting horses, experimentally, is by way

of the nasal passage, and the nasal mucous of infected horses is invariably infectious while a slight catarrh of the upper respiratory tract is usually present.

It appears that, in the horse, there is more than one type of meningo-encephalo-myelitis, the most important and the most specific being "Borna" disease, which was first noted in Germany many years ago. In recent years, the amount of literature published on this disease has been extensive and workers on it may be divided into three schools:—German, French and American, although in other countries there are copious records of investigation.

The workers in France have described a disease practically identical with Borna "disease but differing from it in certain important characters.

In "Borna" disease the important lesions are confined to the central nervous system. There is a slight hyperaemin of the meninges of the brain and spinal cord, and an abnormal fulness of the meningeal vessels, while punctate hemorrhages are seen throughout the brain substance, more especially in the grey matter. There is a well defined perivascular infiltration around the vessels of the choroid places and the small capillaries and veins, frequently extending through the grey matter.

Hutyra and Marek state that subependymal hæmorrhages may be present, along with slight thickening of the ependyma. Exudation in the meninges is not found, although Hutyra mentions that the choroid plexus may occasionally show gelatinous swelling. There is, usually, a well marked mononuclear cell infiltration in the brain substance with a certain amount of neuroglial proliferation. The most important feature of "Borna" disease is the presence of intranuclear inclusions known as "Joest-Degen" bodies, and according to German workers, these bodies are considered as specific of "Borna" disease as negri bodies are of rabies. As in rabies, they are found most commonly in the hippocampal gyrus. The same authorities state that degeneration of the cell nucleus, such as neuronophagia, is not found in "Borna" disease in Germany.

British workers, while confirming the importance of Joest-Degen bodies, claim that there is a definite destruction of the nucleus of nerve calls in "Borna" disease.

In spite of numerous attempts of workers, in the past, to prove a bacterial etiology, it is generally considered, at the present day, that the various streptococci and diplococci which can be isolated occasionally from the brains of the diseased horses are of no importance.

It has been definitely proved that "Borna" disease is due to a neurotropic virus which possesses a close affinity to the viruses of vesicular stomatitis of the horse, louping-ill of sheep, and epidemic poliomyelitis of the human subject.

The disease in America is analogous to the French form of the disease described by Moussu and Marchand. German workers maintain that the Moussu-Marchand form is quite distinct from their conception of "Borna" disease. although French workers do not accept this opinion. The trend of thought would seem to indicate that the virus producing meningo-encephalitis of the horse may exist in an organotropic form as well as a neurotropic, and accepting this possibility, a ready explanation can be found for the lesions which occur in the Moussu-Marchand type of the disease. These lesions consist essentially of (1) degeneration of the parenchymatous organs along with hæmorrhages. Hæmorrhages also occur in the brain and spinal cord. (2) Joest-Degen bodies are absent. (3) The nerve cells of the brain and spinal cord show definite degenerative changes. (4) It is found difficult on intra-cerebral inoculation, if not impossible in the majority of cases to transmit the disease.

These differences appear on a wide review of literature, to be more of degree than of kind. Many workers mention hamorrhages in "Borna" disease, as well as infiltration and destruction of nerve cells.

Apart from the acceptance of Joest-Degen bodies as being an essentially typical feature of "Borna" disease it appears difficult to accept any definite set of p. m. lesions as being typical.

During the course of one outbreak, hæmorrhages in the brain, spinal cord and parenchymatous organs may be present or absent. The absence of any p. m. lesions at all is, in some cases, the most striking feature of the disease. The lesions in the brain and spinal cord are constant in the majority of the cases, but variations may occur. Infiltrations may be extensive around the capillaries, or absent, in which case the brain substance shows a diffuse mononuclear infiltration. The one definite and invariable lesion seems to be a slight thickening of the meninges, without exudation, and those changes described in the blood vessels. In chronic cases there is a formation of new tissue in the meninges.

Referring again to Hutyra and Marek, there exist a form of enzootic spinal paralysis of the horse which is characterized by multiple capillary hæmorrhages in the various organs, and especially in the spinal cord. It is stated that this disease is caused by streptococci, but in a foot note, some considerable doubt is cast on such an etiology and the question concerning the possible relationship

between this disease and "Borna" disease is raised. If we combine the p.m. pictures of these two diseases as described by Hutyra and Marck, we get an average and accurate picture of the disease which we have been investigating at Multan.

Other disease possibilities have not been overlooked, and the following observations may be made:—

1. Forage poisoning.—The most uptodate information regarding forage poisoning in the horse is published and reviewed by Steyn, D. G. in the Onderslepool Journal of July 1933. There is an essential dissimilarity in symptomology between "forage poisoning" and the disease encountered at Multan, as well as in the lesions of the disease which it is not proposed to discuss here. This dissimilarity is enhanced by the fact that experimental feeding transmissions failed both in horses and in small animals, and further-more, the disease at Multan was not affected in any way by withholding the suspected forage for a period. In typical forage poisoning, symptoms of meningitis, if they do occur, are not predominant and in the few definitely diagnosed outbreaks of forage poisoning on record, the feeding of the suspected fodder or silage to experimental horses succeeded in reproducing the disease in a typical form in the majority of cases. Many outbreaks of so-called forage poisoning are actually outbreaks of Botulism.

The possibility of Botulism has been considered. Here again, the symptomology of pure experimental botulism in the horse is quite different from that seen in equine encephalo-myelitis. Symptoms of lethargy, loss of co-ordination resulting in a staggering gait, hyperaesthesia and muscular twitchings with general symptoms of meningitis are not seen in botulism, paralysis of the tongue, a constant feature of experimental botulism is never observed in equine encephalomyelitis. Neither are tissue hamorrhages nor perivascular infiltrations seen in botulism.

The evidence that the outbreak of the disease in the horses of the 13th Lancers is related to, if not actually identical with, specific enzootic equine encephalo-myelitis as occurring in other countries, may be summarised as follows:—

In all cases of the disease, there is a definite meningo-encephalitis of a disseminating infiltrative, nonpurulent, lymphocytic type with predominating vascular character. The lesions in the nervous system and the general post-mortem features are on a summation of post-mortem protocols, as follows:—

Subacute inflammation of the vessels of the meninges without any exudation, accompanied by perivascular infiltration and frequent hamorrhage. Punctate

hemorrhages are frequent in the brain substance and a typical diffuse mononuclear infiltration of the neuroglia with destruction of the nerve elements is seen.

Similar lesions are present in the spinal cord. Joest-Degen bodies are not found. Hamorrhages occur throughout the parenchymatous organs in the majority of cases, and there may be slight degeneration of the cells of these organs. The lymph glands are occasionally swollen and hamorrhagic. Gastro-intestinal catarrh is noted in a few cases. The bladder contains a thick syrupy urine which is frequently blood-tinged, while the submucosa is studded with hamorrhages. Secondary infection of the bladder consequent on urinary stasis is not uncommon, the end result being, in protracted cases, a thickening of the wall of the bladder by a slow infiltration of granulomatous tissue.

The post-mortem appearances presented are parallel to those encountered in American outbreaks caused, probably, by a virus of the Moussu-Marchand type.

The symptomology of the disease is, on all essential criteria, identical. Recovered cases, in American outbreaks, tend to a permanent disability expressed in terms of altered nervous function and structure. In the one recovered case which was examined, there appeared no clinical disability. Definite proliferative lesions were found in the meninges which were thickened and adherent, as well as in the nerve cells of the brain and spinal cord. Hæmorrhages were persistent in the ependyma, the cerebral cortex, and the internal organs, specially the kidney.

On epidemiological considerations, the parallel is maintained. During the course of an outbreak, one horse or a small group of horses is affected at a time. Additional cases may occur after an interval of 7 to 10 days.

Serum from recovered horses was shown to possess some effect in lessening the severity of the disease when given, during the course of an outbreak, in relatively high dosage, to eases in the prefebrile phase of the disease. Even with the onset of symptoms, serum proved useful in tiding over critical cases to the convalescent stage, cases which might not have been expected to recover normally. A further point of analogy is that in the Moussu-Marchand type of the disease there is a definite prefebrile phase of about four days duration. A similar fact was noted during the outbreak at Multan.

Opinions differ very much as to whether any drug treatment is of avail. In German outbreaks, considerable faith is placed in the intravenous injection of of urotropine in large daily dosage. This drug is excreted by the choroid plexus, and its use appears rational. It was used with a considerable measure of success in the outbreak at Multan.

The disease shows a cyclic incidence.

Further evidence is adduced by the transmission experiments which were attempted, while it is true that rabbits could not be infected by intracerebral inoculation of nerve tissue obtained from diseased horses.

Several guinea pigs died after similar inoculations, without, however any symptoms of the disease in question. (The nerve tissue of these cases has not yet been examined). This finding corresponds with that of workers in America, who failed, in the majority of cases, to reproduce the disease in rabbits, and found that guinea pigs were more suitable for experimental inoculations, although these animals showed no typical symptoms of the disease, apart from lesions in the brain after death.

It has lately been found that rabbits can be infected by intraocular injection of filtrated brain emulsion, and using the same route, horses can be infected, although not constantly.

The French virus has only rarely succeeded in transmitting the disease when inoculated subdurally, but that it is capable of doing so has been adequately proved.

This fact agrees with our transmission experiments in which we inoculated two horses with brain emulsion from a case of the disease, one subdurally and the other into the temporal plexus with the subsequent development of disease symptoms of a type strictly comparable to the original disease. These symptoms could not be ascribed to errors in technique as a definite incubation period of nineteen days clapsed before the onset of symptoms. In experimental transmission of the disease to horses in other countries, an incubation period of similar duration has been noted.

Our successful result in producing the disease in horse by the intrathecal inoculation of cerebro-spinal fluid is also in accordance with the experience of two German workers.

Lastly although the limited time at our disposal prevented our undertaking any filtration experiments, there is a strong presumption that the disease is of virus origin, an opinion that is strengthened by the fact that no bacteria could be shown, in the few cases when they were isolated from the disease, to possess an etiological role.

The writer is of the opinion that the disease occurring in the horses of the 13th Lancers at Multan is a specific equine meningo-encephalitis probably caused by a neurotropic filterable virus of organotropic type.

## A STUDY OF THE DATA OF MILK YIELDS OF VARIOUS TYPES OF CATTLE OBTAINED FROM THE RECORDS OF THE COVERNMENT MILITARY DAIRY FARMS.

#### III. PREDICTION OF MILK YIELDS.

BY

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(With one text-figure.)

The prediction of milk yields is a frequent necessity in dairy industry. The dairyman wishes to know at times what the probable output of his stock would be during a certain future period so as to ensure himself that his supply would adequately meet any anticipated demand, or that he may make suitable arrangements for the disposal of surplus milk. At other times he wishes to know, when an animal has been in milk for only a short time, what its annual yield would be so that it it is an unconomic animal it may be culled without incurring unnecessary expenditure on it for the whole year. Again, if two animals or two herds of animals have to be compared from data of yields available for different lengths of periods only it is necessary to know what these would have yielded during identical periods of lactation. The knowledge gained in Parts I and II regarding the properties of the lactation curve and the deductions therefrom enable such estimates to be made as are required in the ordinary course of dairy business.

In part I, it was shown how it may be tested whether environmental conditions like feeding, care and management of animals are such as would enable them to produce their best. It was also shown there how an estimate may be formed of the probable yield during a given future period when an animal has been in milk for only a short time. This may be computed either graphically or arithmetically. By an extension of the method it is possible to calculate the yield for a whole lactation. This, however, presupposes a knowledge of the initial rate of milk secretion and the rate at which it falls off. But often it is necessary to calculate the lactation yield when one or both of these are unknown. This is especially required in the official testing of herds. The tester is able to visit the Farms in his jurisdiction only periodically and he has to form an estimate of the milk producing capacity of the animals from the short time performance he records. This makes it essential that a method should be found by which it should be possible to estimate the lactation yield from the rate of yield at any stage in the lactation.

The rate of milk secretion at maximum is often made the basis of estimating the total lactation yield. Prof. Tuff has found that the total lactation yield is 199

times the maximum daily yield. Sanders finds that the ratio of the lactation yield to the maximum weekly yield varies from 24.7 to 29.37 according to the mouth of calving. From the data collected from the records of the military dairy farms the values of this ratio (total lactation yield/maximum weekly yield) were calculated for individual lactations in the case of a herd of buffaloes. The values of the ratio are given in Table I. It will be seen that even when the service period is confined to narrow limits (which more or less defines the length of lactation) the variation in the values of the ratio is large and would introduce appreciable errors when

Table: I.

Ratio between the lactation yield and the maximum weekly yield of individuals in the various service period groups—Buffaloes.

crial	The second second desiration and the second second second	27V 1 V 37 U 13	eriod in weeks.	
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2	23.79	22.08	26.53	39*44
3	16.25	23.92	19-93	29.45
4	28.40	24:35	26:37	19:40
5	25.36	21.60	27.88	27.75
6	24.43		26 59	28.07
7	28.18	38.83	29-95	36.08
8	24.58	22.92	25-32	29-30
9	23.01	22.38	<b>32</b> ·81	32.61
10	26.46	21.76	36.63	25-45
11-	17.52	22.46	20.74	21.08
12		25.28	20.17	22.51
13	21:09	25.70	28.34	00.5
14	25.30	22.44	34.41	26·74 36·07
14	20.03	21:92	23.86	
15	25.46	25:46	21-59	32.63
16	21.47	24 53		35.56
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18	28.96	16:64	*****	27.41
10	교육대원	28.81		20:07
20	29.05	22.70	*****	28.80
21	30.07	27-17	*****	30:77
313	20:45	21.28	44.004	::1-23
23	25 95	33.57	*****	27.33
24	*****	22 11	*****	32:49
25		21.74	*****	26.31
36 27		26.77	*****	25.80
7			*****	22.98
8	-	26.42	******	
9	*****	22.59	*****	
ő l	*****	26.65	- *****	-
ï	•••••	27.81	******	
2	101	30.94	•••••	******
3	*****	29.54		*****
4	******	30.26		*****
5	******	25.68		*****
		28-42	X X X	*****
6	*****	24.52		*****
7	******	24.77		******
8	******	22:40	•••••	*****

applied to individuals. It has been seen in Parts I and II of this paper that the lactation yield is a function of the maximum yield and persistency end that the latter is subject to large variations in the case of individuals. The variation in the value of the ratio is, therefore, obviously due to the variation in persistency. Again, it is not enough if a ratio is found between the maximum and the lactation yield, it should be possible to estimate the latter from the rate of yield at *any* stage of the lactation.

The purpose of testing herds being to obtain a measure of their economic efficiency as producers of milk or fat, what is often required to be estimated is the yield over a definite period rather than the lactation yield. The magnitude of the latter depends largely on the service period, or for that matter on the length of lactation, and is not a reliable index of the milking capacity of the animal. If the yield for a definite length of lactation is to be estimated it can be shown mathematically that the value of its ratio to the rate of yield at any stage in the lactation depends both upon persistency and the stage of the lactation.

Table II shows the values of the ratio between the yield for ten months and the rate of yield at various stages of lactation, from 1 to 9 months after calving, for all values of the rates of decline from 1 to 20 per cent. per month, which range, it has been seen in Part II, includes almost all the animals studied. If the rate of decline is known, the yield for any month of lactation multiplied by the ratio appropriate to that month would give the total yield for ten months. If the rate of decline is not known the best value to take would be that corresponding to the average rate of decline for the breed to which the animal belongs. The estimate can be further improved if use is made of the knowledge (vide Part II) of the relation between persistency and the level of production.

If Table II is examined minutely another interesting fact is brought to light, that would show at what stage of the lactation the estimate formed would be the most accurate when the value of persistency is unknown. It will be seen that if an estimate is made in the first month, the ten months' yield may be anything between 5.27 and 9.61 times the rate of yield according to the value of persistency. In the second month the range diminishes to 6.44 and 9.71, and in the fourth month it is practically zero. At this stage whatever the value of persistency the value of the multiplier is practically the same. In the 5th month the range is narrow and from there it again widens. This fact is illustrated graphically in

TABLE II.

Showing the variation in the ratio of total yield for ten months to the yield per month in the various months of lactation, when the rate of decline per 1b. per month changes from 01 to 20.

Rate of decline per lb. per	William Steph H. P. Sagard Stephen St. Toronto.			Mon	ths of lactat	ion		NO. CO. CO. CO. CO. CO. CO. CO. CO. CO. C
month	1	2	3	4	õ	6	7	8
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20	9·61 9·24 8·99 8·58 8·27 7·79 7·21 6·98 6·77 6·19 6·37 6·19 6·37 6·19 5·54 5·40 5·27	9:71- 9:43 9:17 8:93 8:69 8:48 8:27 8:08 7:89 7:72 7:50 7:40 7:12 6:87 6:87 6:63 6:53 6:44	9:80 9:62 9:45 9:30 9:14 9:00 8:87 8:75 8:53 8:44 8:53 8:44 8:35 8:12 8:12 8:17 7:90 7:86	9·90 9·81 9·81 9·67 9·61 9·56 9·51 9·48 9·42 9·42 9·42 9·44 9·44 9·46 9·46 9·50 8·55 9·60	10°01 14°02 10°03 10°07 10°10 10°16 10°21 10°27 10°34 10°42 10°52 10°61 10°72 10°83 10°96 11°11 11°22 11°38 11°54 11°73	10·10 10·22 10·34 10·48 10·62 10·78 10·95 11·32 11·52 11·52 11·78 11·96 12·20 12·47 13·03 13·29 13·62 13·62 14·33	10°21 10°42 10°65 10°91 11°16 11°45 11°74 12°05 12°38 12°78 13°10 13°49 13°90 14°34 14°79 15°30 15°76 16°31 16°31 16°39 17°50	10·32 10·64 10·97 11·36 11·74 12·16 12·59 13·55 14·62 15·21 15·83 16·49 17·19 17·95 18·68 19·52 20·42 21·38

These are the most appropriate figures for use with specially bred Indian cows.

cross-bred cows and buffaloes. ordinary Indian cows.

#### FOOT-NOTE TO TABLE II.

Note 1.—By means of this table the total yield for ten months can be estimated from a knowledge of the yield for any month of lactation. This is done by multiplying the known monthly yield by one of the ratios tabulated. The appropriate ratio to take depends on the stage of lactation (i.e., 1st, 2nd, 3rd, etc., month after calving) and the rate of decline. If the latter is known the ratio is obtained by running the eye down the column for the month of lactation, to which the yield pertains, and against the value of decline. If the decline is not known this may be assumed to be the average for the class of animals to which the herd belongs. This is '08 for specially bred Indian cows, '10 for cross-bred cows and buffaloes and '12 for the ordinary Indian cow. The values for these classes are printed in

Example-

If a buffalo yields 400 lb, of milk during the third month of lactation how much will she yield in ten months? For buffaloes the rate of decline is 10. Against this value the ratio is 8°53 in the column for 3rd month. The yield for 10 months is therefore 400 853 or 3412 lb. If she yields 300 lb. in the 6th month her total yield for 10 months will be 300  $^\circ$  . 11:52 or 3456 lb.

Similarly if an ordinary Indian cow yields 400 and 300 lb. in the third and sixth month of lactation, her ten months' yields will be 400 × 835 or 3340 lb. and 300 × 1196 or 3578 lb. respectively.

Note 2 .- In estimating stage of lactation, the month of calving should be omitted. Thus if a cow calves in June, July is her first month of bactation, August second month, September third month

Note 3 -The peak of lactation is generally reached between the 3rd and the 6th week after calving, and if the yield for these four weeks is known, yield for a lactation of 300 days is approximately obtained by multiplying the average drily yield for these four weeks by:

223.8 in the case of specially bred Indian cows.

209.4 in the case of buffaloes and cross-bred cows.

1971 in the case of ordinary Indian cows.

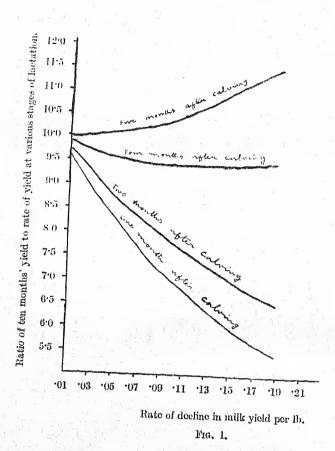


Figure I where the values of the ratio are plotted against the rates of decline for the 1st, 2nd, 4th and 5th month of lactation.

The above fact, namely, that the 4th month of lactation is the best time for conducting a test, may be verified practically. It is obvious that the estimate based on that month's yield would be the best which bears the strongest relationship to the total yield. The relationship is measured by the coefficient of correlation between the two. Ten months' yields were found in the case of 157 cross-bred cows and the correlation of this was found with the yield for each of the months of lactation from 1 to 9. The correlation surfaces for the 1st, 2nd, 4th, 5th and 8th month are shown in Tables III to VII and the coefficients for all months are entered

# TABLE III.

Correlation between rate of yield one month after outving and total yield for 10 months.

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Correlation between rate of yield 2 months after calving and total yield for 10 months.

400-10. units.									or ne ro	0-1D- u	nits.	rade of yield in 100-lb. units. (Class mid-point.)	nid-poi	nt.)				
mid-points.	25.55	3.5	2.5	تن تن	6.5	7.2	 	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	Total
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TABLE V.
Correlation between rate of yield 4 months after calving and total yield for 10 months.

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TABLE VI.

Correlation between rate of yield 5 months after calving and total yield for 10,

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# TABLE VII.

Correlation between rute of yield 8 months after calving and total yield for 10 months.

10 months' yield in 190-lb. units.	<b>a</b>	x = X			heed	Rate of yield in 100-lb. units.	yield iı	n 100-11	o. unifs		ss mid.	(Class mid-point.)					-	ŧ
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Correlation between 300 days' yield and rate of yield at various months after calving.

Table VIII.

M	Months	after	ealv:	ing	Rate of yield	300 days' yield	Coefficient of correlation
1	•		× ,		946-88	7039·S	8722
2					902-87	7039.8	•9082
3	• ,		•		834:72	7039.8	•9292
4			•	, •	754.46	7039 8	.9383
5					679.94	7039.8	<b>'93</b> 56
6	·	•	•		617.52	7039-8	9162
7		-	•		565.29	7039.8	·8714
8	- 1	•	•		514.97	7039-8	*8362
9	: •				462.74	7039.8	•7191

in Table VIII. This table supports the conclusions arrived at above. The value of the coefficient rises to a maximum in the 4th month and then declines showing that the strongest relationship is at this stage, although the differences between successive coefficients do not appear to be statistically significant. From this table it is seen that while the best estimate is obtained by making a test in the 4th or the 5th month estimates at any time between the 3rd and the 6th month would ensure reasonable accuracy. If a tester visits a farm three times a year at equal intervals fairly accurate estimates could be made of the milking capacities of all the animals in the herd.

#### COMPARISON OF ANIMALS WITH DIFFERENT LENGHS OF MILKING PERIODS.

Sometimes it becomes necessary to compare the relative values of two animals or two herds of animals when only a portion of their lactation is known. When the yields over isolated periods at different stages of lactation are the only data available, the yields for ten months can be computed in each case by means of the ratios in Table II and the animals compared. If, however, records are available showing the total yields from calving to different lengths of milking periods it is necessary to know what the animals would have yielded in the same number of days. It is known that the yield is not perpertional to the number of days the animal is in milk. Thus if an animal remains in milk for 400 days her 300 days' yield is not \( \frac{1}{2} \) of the yield for 400 days. It is some other fraction depending upon

the rate of decline. It is possible mathematically to deduce this relation from the equation to the lactation curve. It is convenient to reduce all yields to a standard length, preferably 300 days which is the average lactation length. Table IX shows

TABLE IX. Correction for length of lactation taking 300 days as standard.

Leng	gth o	f lact	a-	When	n rate of decline per m	onth per 100 lb mil	k is
-	tio	n.	-	(j.()	8·0(a)	10.0(2)	12-0(c)
200	•			1.368	1:339	1.300	1.269
210		4		1:315	1.289	1.257	- 1:230
220		·		1.268	1:246	1.217	1.194
230				1:224	1.204	1.180	1.161
240				1.184	1.168	1.148	1.132
250	+	•		1.147	1.147	1.119	1.105
260	*	*		1.113	1.103	1.091	1.081
270			·	1.081	1.674	1.065	1.058
280	•	<b>V</b> .	·	1.052	1.047	1.042	1.037
290	•	•		1.025	1.023	1.021	1.018
300	•			1.000	1.000	1.000	1.000
310	4 .	•		9766	9788	9813	.9833
-	•	#		•9480	9590	9642	.9881
330		· <b>V</b>		19342	* 9404	9475	19537
140		4	· ·	·9145	-9230	9324	9401
350		-		·8964	. 9065	.9181	•9276
360	• '			-8792	-8913	.9048	9158
370	<b>4</b>		- 1	*8630	·8768	·8921	9048
80	-	* ,		*8478	*8632	·8804	*8943
90				·8332	8487	·8692	*8847
100	•			8194	8370	8584	*8762

<sup>(</sup>a) These figures are to be used for specially bred Indian cows.
(b) , , , , cross-bred cows and buffaloes,
(c) , , , , ordinary Indian cows.

#### FOOTNOTE TO TABLE IX.

Example. The following example illustrates the use of the table. Administration Report on the

by what figures a given yield should be multiplied in order to reduce it to a yield for 300 days. An example is given at the foot of the table which explains how the Table may be made use of in practice.

#### SUMMARY.

How yields may be predicted from known portions of the lactation are dealt with. A complete lactation from an incomplete one may be predicted either by computing the area under the curve or by arithmetical calculation by multiplying the yield at maximum successively by the rate of decline, and summing up the products. This requires a knowledge of the rate of decline, and the average value obtained for the decline in parts I and II may be utilised. A ready reckoner table (Table II) is given by means of which complete lactation may be obtained by multiplying the monthly yield at any stage of the lactation by an appropriate ratio given, in the table.

The peak of lactation is generally reached between the 3rd and 6th week after calving, and if the yield for these four weeks is known, the yield for a lactation of

Military Dairy Farms, Northern Circle, for the year 1932-33, Table 13, page 21, shows the lactation yields as under:—

Breed	Av. milk yield in lb.	Average number of days in milk
Ferozépore Pedigree	7,912	390
3/4 Foreign	6,885	335
5/8 ,,	6,851	310

In order to see how these herds compare it is necessary to know how much they yield during the same period. The figures in the table show how lactation yield of any length from 200 to 400 days may be converted into yields for identical periods, namely, 300 days. For conversion that figure should be employed which is below the rate of decline appropriate to the breed to which the herd belongs. Thus for the Pedigree Sahiwal it has been found that the rate of decline is nearly 8 per cent., and the correction factor is obtained from the column headed 8.0. Similarly for the cross-bred the correction is obtained from the column headed 10.0. The yields for 300 days will be:—

Ferozepore Pedigree	7,912 ×	8,632 or 6,830 lb.	
		9,400 or 6,472 lb.	
5/8		0.219 on 6 frug 11.	

(N.B.—In the case of 3/4 foreign where the number of days is 335 the average of the figures against 330 and 340 is taken.)

When the yields are thus expressed the order of merit of the herds can be readily appreciated.

300 days is approximately obtained by multiplying the average daily yield for these four weeks by:-

223.8 in the case of specially bred Indian cows.

209.4 ,, ,, of buffaloes and cross-bred cows.

197·1 ,. .. of ordinary Indian cows.

An examination of values of the ratio in Table II shows that the best time for conducting a short time test is the 4th month. Reasonable accuracy is, however, obtained by using any yield between the 3rd and the 6th month of lactation.

A Table is given by means of which yields for different lengths of lactation may be corrected to a standard length for purposes of comparison.

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#### A SHORT HISTORY OF SURRA TREATMENT IN THE PUNJAB.

 $\mathbf{B}\mathbf{Y}$ 

#### THE CIVIL VETERINARY DEPARTMENT, PUNJAB.

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#### INTRODUCTION.

The history of treatment of surra in India dates as far back as 1893, when Lingard, Imperial Bacteriologist at Poona, introduced a form of treatment for equine surra with certain arsenical preparations, notably atoxyl, without any great success.

Subsequently, Holmes and Cross [1908], working at the Muktesar Laboratory, tested the efficacy of a considerable number of drugs, in addition to those used by Lingard, in the treatment of surra as it affected both equines and dogs. Of the drugs tested by them, mention may be made of atoxyl and its proprietary modification known as soamin, orpiment, arsenious acid, and certain antimony and mercury preparations. A perusal of the records of their trials shows that while in a proportion of the cases treated by them the results were encouraging, the number of failures was by no means small, while in a large percentage of cases, where definite recoveries were believed to have been obtained, relapses occurred during a post-treatment observation period ranging from 4 to 6 weeks.

Edwards [1926], working in the same laboratory, carried out what would appear to be an intensive series of trials upon the comparative trypanocidal value of potassium and bismuth salts, tryparsamide and naganol and was eventually led to formulate the so-called intravenous-intrathecal form of treatment, to which a detailed reference will be made later in this paper. As to tryparsamide and bismuth compounds, it may be stated at once that, on the recommendation of Dr. Edwards, the efficacy of these drugs was then tested upon three artificial and two natural cases of surra in the Punjab, but the results obtained cannot be declared to have been satisfactory. A full dose of tryparsamide followed by a dose of tartar emetic failed to sterilize the blood during a post-treatment observation period of 6 days, whilst the administration of bismuth salts was followed by inappetence, dullness and congestion of the lungs.

The work briefly alluded to above, although mainly carried out elsewhere than in the Punjab, is nevertheless of sufficient historical interest to deserve brief mention in this paper, inasmuch as the results of these trials very clearly demonstrate the futility of efforts directed to curing surra by means of arsenical compounds, and,

as will be noted later the two drugs which gave greatest promise in the trials carried out in the Punjab were Tartar emetic and Naganol, neither of which, as is well known, is an arsenic derivative.

#### TREATMENT OF SURRA BY MEANS OF TARTAR EMETIC.

The necessity for instituting an intensive series of trials upon the possibility of successfully combating surra in the Punjab was realized as far back as in the year 1906, when an officer, with the designation Camel Expert, was specially appointed by Government, with his headquarters in the Punjab Veterinary College at Lahore, for the purpose of investigating the problem, particularly with reference to the possibility of devising suitable measures against the disease as it affected the camel population of the province. This species of animal has long been extensively used for purposes of transport by the civil population and particularly in connection with military operations on the Frontier, the mortality in these animals on account of surra being some times as high as 90 per cent. In 1908, the headquarters of this officer were transferred to Sohawa of the Jhelum district, Northern Punjab, a typical camel breeding country with suitable pasturage and central for the carrying out of investigational work into diseases of camels.

Leese, who held charge of the Sohawa Laboratory as Camel Expert during the first five years of its existence, carried out an extensive series of trials in the treatment of camel surra by means of soamin, tartar emetic, arsenious acid and sodium arsenate, used singly or in combination. In a report subsequently published by him he claimed 62 per cent. of recoveries by the application of the socalled "668" method which involved the combined use of the first three of these drugs mentioned above. As pointed out by Cross, however, shortly after he assumed charge of the Sohawa Laboratory in 1912, the methods of treatment employed by Leese were essentially modifications of those previously used with a certain amount of success by Cross himself and Holmes in the treatment of a large number of artificially infected cases of equine surra at the Muktesar Laboratory. It was further pointed out by Cross that the percentage of recoveries obtained by Leese could hardly be regarded as a true indication of the efficacy of this method of treatment in that the percentage was based by him upon the results obtained in only 9 animals. Eventually Cross discontinued Leese's line of investigation and initiated a series of experiments which had for their object the development of a method of single-drug therapy suitable for adoption by field workers in India, and the drug which was afterwards found by him to fulfil this object was a 1 per cent. solution of tartar emetic injected on alternate days, in doses of 50 to 200 c. c., covering a period of 37 days. This method, which constituted at that time the routine treatment for camel surra, was practised in the case of these animals with a considerable degree of success by the military authorities both in and outside India. By arrangement with the military authorities, numerous batches of infected camels were successfully treated at Sohawa by Cross and his assistant Chaudhri Kahan Singh up to the time of its final adoption by R. A. V. C. officers.

A small amount of preliminary investigational work had been done by Cross on the efficacy of tartar emetic in the treatment of equines also but amongst artificially infected cases only.

On Cross's retirement in 1923, there being no Indian Veterinary Service Officer available, his Assistant Veterinary Inspector Chaudhri Kahan Singh, a graduate of the Punjab Veterinary College, was placed in charge of the Sohawa Laboratory, which was then placed as a district charge under the direct supervision of the Chief Superintendent, Civil Veterinary Department. Henceforward the Sohawa Laboratory was to become more closely identified with the needs of the districts. The time was ripe for pressing on with propaganda amongst camel breeders to bring their surra cases to veterinary hospitals for treatment. Horse breeding in many areas of the province was in a very precarious condition about this time owing to the heavy incidence of surra and the only advice that could be then offered to breeders was to shoot their sick ponies.

In earlier trials, Kahan Singh had tested the efficacy of the "Cross method" upon 14 artificial cases of equine surra and obtained what appeared complete recovery in as many as 13 of these. In view of these unexpectedly satisfactory results, it was considered desirable to try the same treatment in cases of natural surra. The Sheikhupura Veterinary Hospital located near the Deg Nullah, the worst surra zone in the Punjab, was selected for the purpose. Twenty-six ponies suffering from surra in this locality were subjected to this treatment, but only five cases were cured. Although these results could not be regarded as very satisfactory, the form of treatment, nevertheless, gave undoubted promise of success if carried out under conditions that would ensure adequate feeding arrangements for the animals during their course of treatment. As a matter of fact, the treatment had to be carried out under considerable disabilities consequent upon a lack of staff and stabling, whilst the owners themselves, whose previous experience had shown them the futility of all curative measures against the disease, were not willing to provide even the feeding for their animals while under treatment. The District Board, which was approached for funds to supplement the feeding arrangements by owners, would not agree to a policy of spoon feeding. However, in the surra season of 1924, the value of this method was further tested, under somewhat better conditions, upon 96 cases of natural surra, all belonging to private owners. at four different centres in the Punjab, resulting in 49 cures. While these results were encouraging, the drug was found too irritant and drastic in effect to be suitable for treating debilitated animals and in many instances the treatment resulted in the animal refusing its food altogether. This was serious inasmuch as many of the cases were already emaciated when they arrived for treatment. Furthermore, the "Cross method" of treatment by tartar emetic alone was hardly suitable for application in cases of massive infection, as on account of the drastic action of the drug it not infrequently resulted in the death of the host consequent upon the occlusion of blood capillaries by dead trypanosomes or the liberation of toxic decomposition products.

In view of the irritant effects of potassium antimony tartrate, tartar emetic, Colonel E. E. Martin, Deputy Director of Veterinary Services. Northern Command, suggested some trials with the sodium salt, particularly in the case of old and debilitated animals. The relative efficacy of these two salts was, therefore, tested upon 50 cases of surra, 24 of which were treated with the potassium and 26 with the sodium salt. Eighteen animals of the first category were cured, one relapsed and five failed to withstand the action of the drug. The latter were later treated with naganol-emetic combination (infra). Of the 26 animals treated with sodium antimony tartrate, 15 were cured, 2 relapsed and 2 died, whilst the remaining 7 could not withstand the action of the drug and were later treated either with naganol alone or by the combined naganol-emetic method (infra). As will be noted from these results, the percentage of recoveries obtained with the potassium antimony tartrate was slightly higher than that obtained with the more costly sodium salt. The results of these trials further demonstrated the inadvisability of using either of these drugs in the treatment of old and emaciated animals.

#### TREATMENT OF SURRA BY MEANS OF NAGANOL ALONE.

In the Punjab, the earliest trials with naganol were carried out in 1924, when a small supply of the drug was obtained and tested upon 6 animals artificially infected, recovery being obtained in 4 cases. In 1925, the manufacturers very kindly supplied the Sohawa Laboratory with 800 grammes of the drug for further experiments and its efficacy was tested upon 10 cases, with a successful termination in five. The results of these trials demonstrated the superiority of naganol over tartar emetic in three important respects. In the first place, the treatment was simpler and entailed a less prolonged course of treatment than the "Cross method." In the second place, the drug lent itself for injection by the intravenous route without resulting in abscess formation, as is not infrequently the case when tartar emetic is used in this manner. Lastly, the drug could be safely injected even into animals showing swarming trypanosomes in blood smears. Furthermore, an improvement in the condition of the affected animal was noticeable within a few days of the commencement of the treatment and its appetite remained unimpaired.

In 1926 when the drug was placed on the market a sufficient stock of the drug was obtained for further experiments which were conducted in two forms: (1) naganol alone; (2) naganol plus tartar emetic. In the present section, it is proposed only to deal with the results obtained with the first form of treatment, whilst a separate section will be devoted to a consideration of the results obtained by the application of the naganol-emetic combination.

A total of 10 animals were treated with naganol alone. These comprised 5 camels, 3 ponies and 2 dogs and recovery was obtained in all excepting 2 ponies, in which, in spite of the treatment, the disease progressed to a fatal issue. Three of the camels had been previously treated with sodium antimony tartrate, but had failed to withstand the drug.

While these trials were in progress, Edwards [1926] at Muktesar, introduced a form of treatment with naganol which had for its object the sterilization of the cerebro-spinal system simultaneously with that of the circulating blood, it being claimed that this method of using the drug was particularly indicated in the case of animals in an advanced stage of the infection. The routine method, as developed by Edwards, was as follows:—

I. (a) Inject 50 c. c. of a 10 per cent. solution of "Bayer 205" intravenously, per 1,000 lbs. body weight.

(If the horse is in very low condition, administer one-half this dose, and give the remaining half a week later, or as soon as the horse shows appreciable improvement in condition.)

(b) Inject 20 c. c. of a 0.1 per cent. solution of "Bayer 205" intrathecally, per 1,000 lbs. body weight.

(The solution for intrathecal injection is made by taking I c.c. of the solution for intravenous injection, and adding sterile water or normal saline solution to make up 100 c.c. of dilution).

II. Fifteen days after I. Repeat I (b) only.

III. Fifteen days after II. Repeat I (a) (i.e. full intravenous dose), and I(b).

No further medical treatment was required. Careful attention must be given to restoration in bodily condition, by good feeding and grooming combined with exercise.

This method was given a fairly extensive trial at the Sohawa Laboratory, but had eventually to be abandoned, in view of the impracticability of carrying out the technique with the aid of the small staff usually available for work of this kind under field conditions in India.

Some trials have also been carried out to test the efficacy of the single injection method of treatment with naganol for surra infected camels. A total of 140 camels were treated by a single injection of 4 grammes of naganol, which resulted in 16 relapses being reported within one month. On account of the more favourable results claimed for this method by the military authorities a further extended trial is contemplated.

TREATMENT OF SURRA BY MEANS OF NAGANOL COMBINED WITH TARTAR EMETIC.

It is desirable to mention at this stage that a certain amount of overlapping was unavoidable between the different categories of experiments reported upon in this paper. The order in which the chapters are arranged does not bear any definite relationship to the chronological sequence in which the actual methods of treatment were adopted from time to time although in a report of this kind which purports to be a historical résumé of surra treatment in the Punjab, it would have been in accord with the customary procedure to deal chronologically with the developments that led to the present day method of treating surra in this province. On the other hand, there is an obvious advantage of consolidating and grouping these developments under appropriate headings so as to bring into prominence the results obtained with any one of these forms of treatment.

Initially, the object of the trials with the naganol-tartar emetic combination was to test the possibility of reducing the cost of treatment with naganol alone. In the preliminary trials, 3 camels and 8 equines were subjected to this form of treatment and recovery was obtained in 6 of these cases.

The foregoing results seemed sufficiently encouraging to warrant some further trials with this form of treatment on a larger scale. Accordingly, the curative value of the treatment was tested upon 17 ponies and 26 camels, with the following results:—

- 1. Fourteen out of the 17 ponies were cured and discharged, three of these having been previously unsuccessfully treated with naganol alone.
- 2. Out of the 26 camels, 22 were cured, 1 relapsed 25 days after the completion of the treatment and the remaining 3 died. The recoveries included 8 animals that had been previously unsuccessfully treated by the "Cross method". The following conclusions were arrived at as the result of these experiments:—
  - (1) There was a possibility of chronic cases being cured by this method; (2) there was no risk of untoward effects when the combined drugs were administered during the paroxysm of the disease; (3) the beneficial

effects of the drugs, as evidenced by an improvement in the general condition of the animals, were noticeable even during the progress of the treatment.

While the foregoing experiments were in progress at Sohawa the combined naganol-emetic treatment was also being subjected to an official trial at the Sheikhupura surra centre. Thirty-four ponies were selected for this trial and recovery was obtained in as many as 11 animals, although the majority of the cases were in an advanced stage of the disease. These results were distinctly encouraging so that attention was henceforward exclusively confined to perfecting this method of treatment.

In 1927-28, the efficacy of this method was further tested on 142 camels, and out of these 137 were cured, 4 died while under treatment and 1 was destroyed on account of a fractured leg. Reports received 3 months later from the owners of these 137 camels showed that, with the exception of one animal that had died from causes other than surra, all were alive and performing normal work.

In the same year, 114 ponies at four centres were likewise subjected to this method of treatment, and out of these, 100 were discharged as cured.

The year 1928-29 was a comparatively dry year and cases of surra were, therefore, not so numerous as during previous years. Only 31 camels and 109 ponies arrived for treatment at different centres in the province, and out of these 27 camels and 102 ponies were cured and discharged, 2 camels died while under treatment and 7 ponies were sold by their owners before the completion of the treatment. According to reports received at a later date from their owners, all of the 129 animals discharged as cured were alive and the majority had gained in weight.

In view of equally successful results from a further series of experiments on the same lines, the following method of treatment was ultimately drawn up for routine use under field conditions in the Punjab and is still being adhered to.

Intravenous injections to be carried out at intervals of four or five days in the following approximate dosage:—

	c.c.
1 per cent. solution of naganol	100
l per cent. solution of tartar emetic	100
1.5 per cent. solution of tartar emetie	100
1.5 per cent. solution of naganol	100
2 per cent. solution of tarter emetre	100
2.5 per cent, solution of tarter emetic	100
2 per cent, solution of nagenol	100

The increase in the dosage is not a fixed one, but depends upon the condition of the animal, and symptoms. if any, caused by the previous dose. The full course of treatment occupies approximately one month.

During the years 1929-34, a total of 13,561 animals were treated at surracentres by the naganol-emetic method as outlined above, with the following results. It has been recently computed that 57 horses and 53 camels were definitely traceable as relapsed cases out of the total number treated.

Surra	eeason	Number of unit treated	nals Nun	iber of cure	Number of that died under tre	wh	iile	Remarks			
1929-30	Ş	Camels .	99 Cam	els	. 93	Camels	4	6	-	A STATE OF THE PERSON OF THE PERSON	هر پس شبه استان دادها
	. 5	Horses . :	386 Hor	68	. 355	Horses		31			
1930-31	(	Camels .	170   Cam	els )	} .1,751	Camels	٠,	15			
	(	Horses . 1,	135 Hora	es )		Horses	,	139			
1931-32	<b>\{</b>	Camels . (	76 Cam	els )	} . 2,379	Camels		262			
		Horses . 1,8	65 Hors	és S		Horses }					
1932-33	5	Camels . 6	16 Cam	els )	) . 1,914	Camels		610			
	3.	Horses . 1,5	10 Hors	es J		Horses		212			
2000 04	(	Camels . 3,6	23 Cam	əls	. 3,584	Camels		40			
1933-34	- ' (	Horses . 2,7	81 Hors	es -	. 2,595	Horses		185			

In the case of those animals which were discharged as cured, reports received from their owners showed that they were practically all alive and in good condition: some months after treatment and as a further check on the results of surra treatment at the different surra centres regular inspections of cured cases are being made by the Director of Veterinary Services in the course of his tours.

In view of these strikingly successful results, the naganol-emetic treatment had rapidly gained in popularity and in the surra season of 1933-34, as many as 39 centres for surra treatment were open in different parts of the province.

It will be observed that the schedule of treatment, as outlined above, involves a total of seven injections spread over 31 days. In view of the disadvantages of this prolonged, although otherwise very effective, method of treatment, efforts are being made to reduce the number of injections. Some preliminary trials have already been carried out in this direction and although the results of these offer

some little encouragement no definite conclusion can be arrived at until a large number of natural surra cases have been treated.

At this point, a reference may be made to the view expressed by certain workers that the injection of a trypanocidal drug such as naganol results in the parasites migrating to the cerebro-spinal fluid where they may remain in a sort of safe retreat until the effects of the drug in the circulating blood have passed away. This is believed to occur when naganol is introduced into the circulation, the molecules of this drug being regarded as too heavy to be capable of penetrating into the spinal canal. In so far as the combined naganol-emetic method of treatment is concerned, however, its application was found definitely to bring about a sterilization of the cerebro-spinal fluid as judged by the results of eleven experiments.

During the last surra season ending December 1933, an investigation, on a small scale, into the possibilities of employing naganol as a prophylactic against surra in horses was carried out in some very well known infected villages in the Sheikhupura district. The procedure adopted for the investigation was to select horses for prophylactic treatment from as many different villages and from as wide an area as possible to ensure every likelihood of a sufficient number of the protected horses being exposed to certain infection. In accordance with plan thirteen villages, owning 617 horses, were selected and from these a total number of 52 horses were given protective inoculations, namely, four in each village. The latter received injections according to four categories:—

- (1) Monthly intravenous injections of naganol 3 grammes for the duration of the surra season, six months July to December.
- (2) Monthly intravenous injections of naganol one gramme only for the duration of the surra season, six months July to December.
- (3) Monthly intravenous injection of naganol 3 grammes for the first half of the surra season only, three months July to September.
- (4) Monthly intravenous injection of naganol one gramme for the first half of the surra season only, three months July to September.

As evidence of the prevalence of surra during this season it has been ascertained that as many as 120 out of the 617 horses in these selected villages naturally contracted surra. In one village 32 out of 60 horses contracted surra and in another 23 out of 40.

Out of the 52 horses selected for experiment, seven failed to complete the course of protective inoculations prescribed as their owners were unwilling to continue with the experiment. Of the remaining 45 horses, only one developed surra 28 days after the third monthly injection of neganol 3 grammes. There were also

two doubtful cases which died of paralysis during the period—the first fifty-six days after receiving the third monthly injection of three grammes and the second within two days after the third monthly injection of naganol one gramme.

The remainder passed through the surra season without infection. There has been no definite evidence to show that either of the two cases of paralysis were affected with surra or were adversely affected by the protective inoculations.

If, therefore, the last surra season of 1933 may be regarded as a typical one of surra incidence, the conclusion to be drawn from this investigation is that there is some probable merit in this method of prophylaxis by means of naganol in the dosage employed. It is proposed to carry out a further investigation on the same lines during the surra season of 1934.

### A NOTE ON BOVINE TRYPANOSOMIASIS IN HYDERABAD STATE.

BY

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(Received for publication on 8th February 1934.)

(With Plate XVIII.)

This condition has recently been brought to light in the forest covered district of Asifabad of this State. The two areas affected are in Sirpoor taluq, comprising the viliages of Muthanpet, Lonvelly, Arelli and Kanky; and Luxettipet, Rubanpalli, Dhorpalli and Jogiapet in Luxettipet taluq.

In November 1932, some outbreaks were reported as anthrax from the above areas, but blood smear examination revealed them to be trypanosomiasis.

The general impression in India has been that cattle (especially buffaloes) acted as a reservoir only for trypanosomes. Hutyra and Marek report that outbreaks of an acute form with a high mortality are met with.

Stirling [1927] mentions regarding a severe outbreak of this disease in the western districts of Central Provinces and Berar.

Gopalkrishnan [1927] also refers to outbreaks of this disease in the Central Provinces.

As the condition in Asifabad appeared to be acute, investigation was undertaken during September 1933.

The mortality in the year 1932 is said to be about 300 cattle, mostly bullocks. Two ponies are also reported to have died.

The disease usually occurs during the early winter months when biting flies are plentiful and the plough-bullocks are worked hard.

Blood smears of 297 cattle, 2 ponies and 3 dogs were examined during this investigation.

An important point noted has been that the disease affects mostly (90 per cent.) working bullocks.

The specimens of biting flies collected and identified are as follows:-

- (i) Tabanus tenens.
- (ii) Tabanus bicallosus.
- (iii) Tabanus sp. (?)
- (iv) Musca crassirostris.

Others flies are :-

- (i) Hamatopota sp. (?)
- (ii) Lyperosia exigua.
- (iii) Sp. of Musca and Sarcophaga.

The ticks, collected from animals affected with trypanosomiasis, are:-

- (i) Hyalomma ægyptium.
- (ii) Hæmaphysalis bispinosa.

The blood smears from some of the orushed ticks were found negative for trypanosomes.

#### AN OUTBREAK OF THE DISEASE.

This was seen in Lonvelly on 22nd September 1933. Thirty-seven cases were reported from this village and Arelli (close by) from the middle of September to the end of November 1933. Of these, 12 were found positive for trypanosomiasis by microscopic examination. The following table sums up in brief the attacks and deaths:—

									Number attacked.			Deaths.	
Bullocks	•							(). •		31		28	
Cows .	• •	• •			•					3		3	
Buffaloes	•		•	 •	• .					3		2	
								,	MR encome	************		elementarion and	
						Tot	tal			37		33	

For a period after infection the animal appears to be quite healthy, feeds and works normally. Therefore the owners work them as usual and only become aware of the disease when the stage of fatality sets in.

In the course of routine blood smear examination four cases were detected, one of which showed no signs of illness even the temperature being 101.4° F. but the peripheral blood showed about one trypanosome per ten fields. Another case showed dullness and mucous discharge from the eyes, but ruminated. The temperature was 105.4° F. and the blood revealed 20 to 30 trypanosomes per field.

The apparently healthy but affected animal is used for ploughing and fatigue following acts as a predisposing cause in bringing about the onset of the fatal stage. In this stage the animal stops to feed and ruminate, is disinclined to move, sways the whole body backwards and forwards and foams at the mouth. The muzzle is dry and hot and there is discharge from the eyes and nose. Dullness and fever are

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apparent. There is hard breathing and excessive urination. Only one case under treatment showed shooting diarrhoea.

Within three to four hours of these symptoms the animal usually goes down, never to get up again. The eyes become semi-closed, coma sets in, the head and neck is either extended forward or turned to the body and the end is soon approached (Plate XVIII).

Some animals which took suddenly ill after the day's plough work were found dead in the fields on the following morning.

Only the first three cases remained alive for a period of about 4 days. It may be their life was extended due to the treatment undertaken. Once the fatal symptoms were exhibited the case did not last for more than 48 hours.

Microscopic examination of blood reveals infection long before the actual fatal stage occurs. Examination of blood smears was carried out at different stages of the disease and it appears that the trypanosomes are rare at first, then they increase, again become rare, and completely disappear about an hour or so after death.

#### POST-MORTEM APPEARANCES.

The cases generally showed lobular pneumonia with adhesions to the pericardium and thoracic wall. The bronchial lymph glands showed deep congestion. Petichæ were noted throughout the serous membranes, and on heart and abomasum. In some cases only petichæ were observed.

The smears of heart blood, lung, spleen, liver, kidneys, cerebro-spinal fluid and brain, taken after death, did not reveal trypanosomes.

#### HISTO-PATHOLOGICAL EXAMINATION OF THE TISSUES.

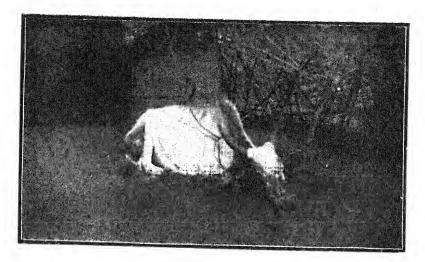
#### "1. Lungs and bronchial lymph glands.

Lungs reveal a very acute type of pneumonia and large number of necrotic foci. The interlobular septa show marked distention due to accumulation of inflammatory exudates. Numerous diphtheroids were seen in the necrotic zones.

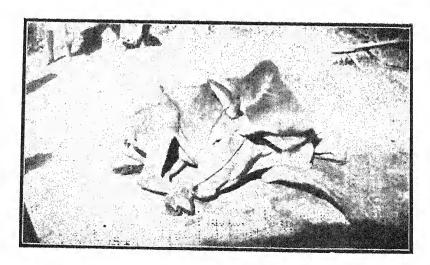
The bronchial lymph glands show intense congestion only.

#### 2. Brain and spinal cord.

The piece of brain and spinal cord do not show any abnormality. No surra parasites were seen in the sections prepared from these tissues."



Case No. 6. This cow died three hours after the photograph was taken.



 $\it Case~No.~12.~$  This bullock died an hour after the photograph was taken.



Treatment was tried on three animals in the fatal stage, giving 25 c.c. of a 10% solution of Bayer 205 intravenously for an adult (approximate body weight being 500 pounds) with the result that only the trypanosomes disappeared from the peripheral blood but the patients succumbed.

Four cases detected in the early stage by microscopic examination recovered with the above treatment.

A buffalo calf received 50 c.c of 1% solution of Bayer 205 intravenously. Sixteen hours later trypanosomes were still present in the peripheral blood and an injection of 10% solution was given and the animal completely cured.

It was noted that after treatment with 10% solution of Bayer 205 intravenously, the trypanosomes disappeared from the peripheral blood in about 16 to 18 hours after injection.

#### SUMMARY.

- 1. The existence of bovine trypanosomiasis in Asifabad district of this State was recorded for the first time in November 1932. The attacks were so very acute that the outbreaks were mistaken for anthrax.
  - 2. The 90% of attacks are in bullocks, and mortality is cent. per cent.
  - 3. The species of flies and ticks collected from the affected areas are:-
    - (i) Flies of Tabanus species, and Musca crassirostris.
    - (ii) Cattle ticks Hyalcuma agyptivm, and Hamaphysalis bispinosa.
  - 4. Symptoms and post-mortem lesions are dealt with.
- 5. Bayer 205 in 10% solution given intravenously is found effective before the fatal onset occurs.

Further work to find out whether the species of trypanosoma concerned is *Trypanosoma evansi* or some other species shall be taken up shortly in collaboration with the Imperial Institute of Veterinary Research, Muktesar.

Acknowledgments. I take this opportunity to express my thanks to all concerned, especially to Mr. Aliuddin Ahmed, Assistant Veterinary Surgeon, who worked hard in this connection.

My thanks are also due to the Director, Imperial Institute of Veterinary Research, Muktesar, for his kindly examining the materials submitted.

I am grateful in particular to Mr. B. K. Badami, Officiating Director, Civil Veterinary Department, Hyderabad-Deccan, for the various helpful hints that he gave.

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### ON THE NEMATODE CAUSING STOMACH TUMOURS OF THE INDIAN CROCODILE, CROCODILUS PALUSTRIS.

BY

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(Received for publication on 10th July 1934.)

(With four text-figures.)

Through the courtesy of Major C. S. Nuttall, the Officer Commanding, Military Veterinary Hospital, Jullundur, Punjab an opportunity was afforded to examine worms from a tumour found in the stomach of a crocodile shot at Jullundur. worms on examination proved to be Mullicacum agile Wedl, 1862. Apart from the morphological interest of the parasite, particularly in respect of its alimentary canal, which led Baylis [1923] to create a new genus for it, it possesses considerable interest from the point of view of geographical distribution. Up to the present moment it has been recorded only from Egypt. this being therefore the first occasion of its record from a country outside Africa. Its original description was given by Wedl as early as 1862. Subsequent to this date Baylis [1923] published a short description of this parasite. It appears from both these accounts that the material at the disposal of the authors was very scanty. The present opportunity is therefore taken to supply some more data regarding the parasite based on the observations of about half-a-dozen males and an equal number of females at various stages of development and to revise in some detail the previous description of this species.

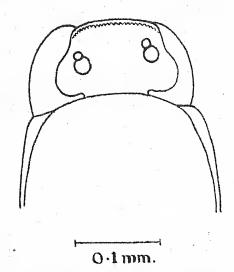
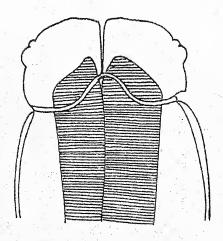


Fig. 1. Multicecum agile. Head, dorsal views



0.1 mm

Fig. 2. Multicæcum agile. Head, lateral view.

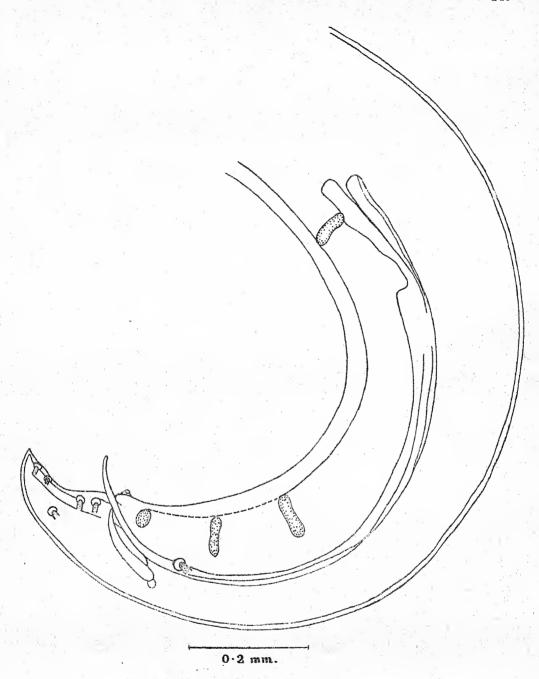
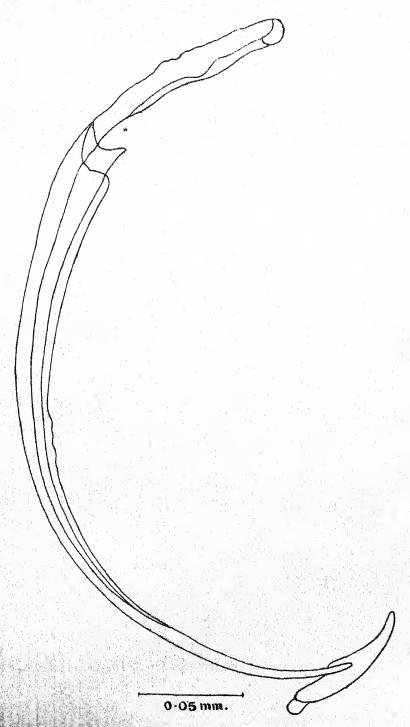


Fig. 3. Multicacum agile. Posterior end of male, lateral view.



Fra. 4. Multicacum agile. Spicule with gubernaculum.

#### DESCRIPTION.

The body is straight and cylindrical and the males as well as the females have a tendency to coil their posterior extremity ventrally. The entire surface is covered with striated cuticle. The lips have one large and close to it one small papilla on each side and in addition to these there is a small papilla in the centre of each lip anteriorly. These papillæ on the lips appear to have escaped the notice of the previous authors. Small interlabia are present with well-marked grooves running along the bases of the lips. Dentigerous ridges are also present. Cervical papillæ are very inconspicuous and are situated somewhat midway between the anterior termination of the intestinal cæcum and excretory pore. The excretory pore is slightly posterior to the nerve ring. The æsophagus has a small posterior ventriculus from which arise two anterior and three posterior appendices. The intestine has a finger-shaped process, the intestinal cæcum, passing anteriorly.

The length is 24.5-26.2\* and the maximum thickness which is attained towards the posterior extremity is 0.425-0.53. The diameter of the head is 0.16-0.2. The cuticular striations are 0.003-0.004 apart. The nerve ring, the excretory pore and cervical papillae are situated at a distance of 0.6-0.71, 0.67-0.78 and 0.87-1 respectively, from the anterior end. The intestinal cocum ends at a distance of 1:17-1:185 from the anterior extremity. The length of the œsophageal appendices are: anterior, 0.22-0.37; two outer posterior, 0.48-0.63; and the middle posterior which is the smallest 0.135-0.16. The tail of the male is 0.145-0.6 long, gradually curving ventrally and tapers conically and suddenly into a narrow tip. The caudal also are absent. There are ten pairs of caudal papille: five being preanal and five postanal. Of the preanal series, the first, third, and the fourth are subventral and are fairly close together, the second is lateral. The fifth is situated at a considerable distance from the fourth, more or less close to the anterior extremity of the spicules. Of the postanal series the first, second, fourth and the fifth are subventral while the third is lateral. There is a median papilla on the anterior lip of the cloaca. Although it was not possible to examine the worms ventrally on account of the coiled nature of the tail, the presence of the papilla was observed in the lateral view in all the cases examined. The gubernaculam measures 0.133-0.153 in length and is like a curved rod tapering posteriorly and having lateral expansions beginning a short distance from its proximal end. These expansions also diminish in width posteriorly. At its proximal end it has a solid knob. The spicules are equal, rather sharply bent and measure 104-123 in length. Their anterior fourth is like a rugged rod with a groove ventrally. At their anterior fourth there are very broad chitinous expansions on either side which diminish in width posteriorly and terminate at their posterior fourth. These

<sup>\*</sup> All measurements in millimeters.

expansions form a deep groove on the ventral aspect of the spicules. The posterior quarter of the spicules is a conical rod tapering posteriorly. These details of the spicular structure appear to have been overlooked by the previous authors.

Female. The length is 9.3-28.2 and the maximum thickness which is attained somewhere near the posterior end is 0.23-0.56. The diameter of the head is 0.11-0.225. The cuticular strike are 0.003-0.0035 apart. The nerve ring, the cervical papillæ and the excretory pore are situated at a distance of 0.37-0.66, 0.57-0.98 and 0.47-0.77 respectively, from the anterior extremity. The intestinal cocum terminates at a distance of 0.92-1.47 from the anterior end. The length of the cesophageal appendices are: anterior, 0.15-0.43; two outer posterior, 0.345-0.59 and the middle posterior which is the smallest 0.145-0.23. The tail is 0.22-0.4 long, straight, sharply conical in fully developed forms, but gradually tapering in immature specimens. Situated at a distance of 0.02-0.06 from the posterior extremity is a pair of small caudal papillæ. The vulva is situated at a distance of 12.26 from the anterior end. In the immature forms examined there was no development of the female genitalia nor could the opening of the vulva be detected in them. The vagina measures 1.25-1.8 in length. It may be straight or variously disposed. The remaining portion of the female genitalia are as described by Baylis. The ova measures  $0.063-0.1\times0.058-0.07$ .

Host.—Crocodile (Crocodilus palustris).

Location.—Stomach tumour.

Locality.-Jullundur (Punjab).

The specimens have been deposited in the helminthological collection of the Imperial Institute of Veterinary Research, Muktesar.

It is my pleasant duty to acknowledge my indebtedness to Major C. S. Nuttall for such interesting material, to the authorities of the Asiatic Society of Bengal for the loan of the literature which could not be had anywhere else in India, and to Mr. J. S. Rao, for fair-copying the diagrams.

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# SELECTED ARTICLES

WOOL-GROWTH IN SHEEP AS AFFECTED BY THE CARBO-HYDRATE-CONTENT OF THE DIET.

BY

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AND

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# INTRODUCTION.

It is known that the quantity and quality of wool is affected by the nutritional plane of the sheep. Thus Hardy and Tennyson [1] showed that both the growth and coarseness of wool were greatest in summer or autumn and least in midwinter, and that there appeared to be a close relationship between the thriftiness of a sheep and the quality and quantity of wool it produces. Wilson (2) and Weber (3), as a result of feeding experiments, showed that the plane of nutrition had a marked influence on wool-production. Weber found that sheep full fed on ground corn and alfalfa meal produced more, longer, and coarser wool than those fed on alfalfa meal in such amounts as to cause a slight loss in body-weight.

Owing to the protein nature of wool, nutritional research has been mainly centred on the protein or constituent amino-acids of the sheep's diet. Thus Marston and Brailsford Robertson (4) suggested that dietary cystine was the probable limiting factor in wool-production. In the "Meteor Downs" experiment, Marston

(5) found that bloodmeal (protein of high cystine-content), fed ad lib. to Merino sheep under natural conditions, resulted in a 30 per cent. increase in average fleece-weight. It was found that the in-take of bloodmeal varied with the prevailing fodder conditions, more being taken in the winter months. On the other hand, Fraser and Roberts (6) were unable to influence wool-production by a 50 per cent. variation in the in-take of digestible protein.

Recently Duerden, Bosman, and Botha (7) have shown that a deficiency of phosphorus in a sheep's diet results in a lighter fleece, of shorter length and finer quality, as compared with sheep fed on a sufficiency of phosphorus. The food-consumption of the sheep on the phosphorus-deficient diet was, however, very considerably reduced (8).

The energy value of the diet is known to be of primary importance in body-growth, but the influence of this factor on wool-production has been insufficiently studied. Yet under range conditions of sheep-farming, whether during the cold winters of temperate countries or the periodical droughts of warmer regions, it is likely to be one of fundamental practical importance. For the investigation of this question, a joint experiment between the Rowett Research Institute and the Wool Industries Research Association was planned. One group of ten sheep was fed a maintenance diet adequate in protein, minerals, and vitamins. A second group of ten sheep received the same diet supplemented with maize starch. Any group differences in the quantity or quality of wool grown could therefore be directly attributed to the starch supplement or to its absence.

Methods.—Twenty Cheviot wether hoggs were divided into two groups of ten. the groups being comparable in age, weight, and quality. The sheep were fed indoors, with moss litter as bedding, for a period of 154 days (December 12, 1932 to March 25, 1933). The animals were weighed individually at 7-day intervals. The basal ration was adjusted from time to time to maintain the initial body-weight of the basal group. Identical alterations were made in the rations of the two groups. The details of the dietary plan are shown below:—

	Group. I.	in the second		
Basal ration.	* 1	0		
Linseed-cake me Turnips	al	,	1 lb. )	per sheep daily
Oat straw . Lime 2 2 per cer	t of lineaged	heal feet	1 1b. )	uany
C. L. O. 24 c. cn Water and salt-l	n, per sheep d			

Group II.

Basal ration.

200-500 gm. maize starch per sheep daily. The purity of the starch was tested by determining its nitrogen-content. This was found to be 0.058 per cent. showing that the starch was sufficiently pure for the purposes of the experiment.

At the beginning of the experiment a triangular area was mapped out by iridectomy knife and Indian ink on the skin over the left shoulder of each sheep. The wool within this area was closely clipped with scissors and the sample removed for detailed examination. The fleeces were then shorn with a shearing machine and individually weighed. At the end of the experiment samples were again clipped from the tattooed areas, and the complete fleeces were shorn and weighed. The second samples and second fleece-weights were therefore those of the wool grown during the experiment on diets of known quantity and composition.

#### RESULTS.

Food-consumption.—The total food consumed during the experiment is shown below:—

# Total food consumption during 154 days.

	Gr	oup I	(10 s	heep)		Group II (10 sheep)
Linsced cake			•		749 lb.	As Group I, plus 1,315 lb.
Turnips .	٠	•	. '	•	6,860 lb.	maize starch.
Oat straw			×		770 lb.	

Body-weight.—The effect of the added starch in increasing body-weight is shown in Table I. The percentage increase in body-weight of Group II was ten times that of Group I.

# WOOL-ANALYSIS.

In examining the effects of nutritional influences upon fleece-growth, both the amount of fibre-material produced and the form which it assumes have to be considered. From the sheep-owner's point of view, the financial return is a function of these factors and also of the proportion of non-wool materials in the shorn fleece. In the discussion of experimental results, also, it is necessary to realize that the fibres are not the only products involved in fleece-growth; parts of the 'grease' fraction constitute a like drain upon the activity of the follicles. In so far as these are ancillary to elaboration of fibre, they may not be neglected from any estimate of the requirements of the follicles in respect of any particular nutritional element; so that the estimation of the demands for follicle-growth, derived from analyses of fibre-constituents, may well be regarded as yielding a minimal figure.

TABLE I.

Body-weight in lb.

	GR	OUP I	GROUP II				
Animal	Pre-exptl.	Exptl.	Per cent. change	Animal	Pre- exptl.	Exptl.	Per cent. change
1 3 5	64·5 57·3 64·6	62·5 57·5 67·5	-3·1 +0·3 +4·5	2 4 6 8	66·8 69·0 63·3	77.0 93.5 101.5	
5 7 9	69·6 65·8	83·5 69·5	+20·0 +5·6	10	74·0 74·0	115·0 111·0	+55·4 +50·0
11 13 15	53·6 69·0 53·0	57·0 64·0 55·5	+6·3 7·3 +4·7	12 - 14 16	58·6 51·6 57·0	93·5 93·0 98·5	$+59.5 \\ +80.2 \\ +72.8$
17 20	77·0 57·5	84·0 68·0	+18.3	18 19	53·0 65·0	97·0 104·0	+83·0 +60·0
Mean	63.19	66.90	+583	***	63:23	98.4	+57.2
S. E.	土2.45	±3·20	±2.69		±2.54	±3.35	土6.45

Differences: Pre-experimental, 0.04; experimental, 31.5±4.63; Percentage change 51.4±6.99.

For the present purpose, however, these other products (9,10) will not be considered beyond their general inclusion in the greasy fleece.

(a) Fleece-weights. The sheep were clipped on December 15, 1932, and at the end of the experimental period, on May, 25, 1933, the gross fleece-weights being recorded. From December 6, 1932, until the start of group-feeding (December 22), both groups were kept on the basal ration, hence the fleeces of Group I represent 161 days' growth with the animals on the basal ration, and those of Group II represent 7 days on the basal ration and 154 days with the starch supplement. Any difference in fleece-growth due to this relatively small discrepancy and also to (a) the possible effects of portions of the fibres remaining within the follicles, below skin or clipping level, and (b) any "carry over" growth influence, may be regarded as negligible compared with the amounts grown over such an extended period of experiment.

The pre-experimental fleeces include the changes of the natal coat and the subsequent early stages of maturation; moreover, the length of their growing periods is not precisely known. They are therefore not strictly to be compared with the experimental fleeces so far as fibre-characters are concerned, but the procedure of expressing the differences as percentage changes serves to give measures of the effects of the experimental feeding upon growth.

Throughout the discussion the difference between the experimental growth and that grown previously is expressed as a percentage change on the latter for each individual. In both groups alterations in production occurred in all animals, and the directions and orders of the changes are considered. Also, and for the same reason, the mean values of fibre-length, fineness, etc., for each individual are not weighted in considering the differences between the groups.

The gross fleece-weights of the animals are given in Table II. Before being subjected to the experimental rations the mean fleece-weights of the groups were not significantly different, but the difference in the amount produced during the course of the experiment was significant.

Table II.

Fleece-weights (in qm.)

	Ç	BOUP I	*	*	GRO	OUP II	
Animal	Pre-exptl.	Exptl.	Per cent. change	Animal.	Pre- exptl	Exptl	Per cent. change
1 3 5 7 9 11 13 15 17 20	1,580 1,580 1,500 1,510 1,280 1,350 1,470 1,230 1,750 1,330	494 304 305 605 524 534 223 302 648 662	-68·7 -80·8 -79·7 -53·3 -59·1 -60·4 -84·8 -75·5 -63·0 -50·2	2 4 6 8 10 12 14 16 18	1,730 1,910 1,580 1,500 1,350 1,780 1,380 1,660 1,330 1,280	725 540 1,105 1,170 928 872 858 965 835 1,065	-58·1 -71·7 -30·1 -22·0 -31·3 -51·0 -37·8 -41·9 -37·2 -16·8
Mean S. E.	1,458 ±50.70	460·1 ±51·36	—67·5 ±3·84	THE STATE OF	1,550 ±68 37	906:3 ±59:04	—39·8 ±5·28

Differences of means: Fleece-weights: pre-experimental,  $92\pm85\cdot12$ ; experimental,  $446\cdot2\pm78\cdot26$ ; percentage change,  $27\cdot7\pm6\cdot53$ .

The group receiving the starch supplement, as judged by the fleece-weights, produced almost twice as much as those on the basal ration. The fleeces of Group II, however, contained much adventitious starch, giving them a markedly different appearance and handle, so much so that a trade estimation of yield of clean wool could not be made. The effect on the amounts of wool grown can be judged from the samples taken from the tattooed areas; a rough estimate can be made on the broad assumption that the clean yield of each fleece as a whole was the same as that of the defined sample.

The amounts of wool thus estimated are given in Table III, which indicates that the wide difference in growth upon the two rations is also expressed when the fibre-product alone is considered.

In general appearance the fleeces of the animals in Group II exhibited longer, denser, and thicker staples than those of Group I.

(b) Area samples. The samples were taken from the defined areas on December 6, 1932, and again on May 24, 1933, the wool-growths represented therefore are those of 169 days on the basal ration, for Group I, and 15 days on basal ration, followed by 153 days on the starch supplement for Group II. The samples were exposed for two days in the laboratory atmosphere and weighed in the 'raw' state before being subjected to the standard procedure of analysis [11], viz., washing with three changes of warm benzene, air-drying, and washing with three changes of distilled water. After final drying the clean-dry weights were determined by weighing against a standard sample of known clean-dry weight. To compare roughly the amounts of wool grown, the 'yield' of wool was calculated by expressing the clean-dry weights as percentages of the weights of the raw samples. (It should be noted that the yields before and after experiment are not strictly comparable owing to possible differences in atmospheric humidity obtaining when the samples were weighed.) These are the values given in Table III.

Table III.

Sample yield and estimated weight of clean fleece.

***************************************		GROUP I				, in.	GROUP I		
	Pre-experimental		. Exper	mental		Pre-exp	erimental	Experimental	
Ani- mal	*Sample yield per cent	Estimated clean fleece- weight	*Sample yield per cent.	Estimated clean fleece- weight	Ani mal	*Sample yield per cent.	Estimated clean fleece- weight	*Sample yield per cent	Estimated clean flecce- weight
1 3 5 7 9 11 13 15 17 20	79.0 79.2 80.1 80.7 79.3 79.2 79.4 78.6 76.6	1,248 1,251 1,202 1,219 1,015 1,069 1,167 967 1,397 1,019	74·5 66·9 68·9 75·9 70·2 72·2 66·5 62·4 69·6 73·3	368 203 208 459 368 386 148 168 451 485	2 4 6 8 10 12 14 16 18	78 3 77:3 81:0 74:3 76:4 80:5 78:6 82:0 79:2 76:0	1,355 1,476 1,280 1,115 1,031 1,438 1,085 1,361 1,053 960	56.7 (5.8 47.9 52.8 57.4 62.4 67.9 66.7 55.8	411 855 529 618 533 548 484 655 557 589
Mean	79.2	1,155-4	70.0	326.4	0.	78.3	1,214.9	59.0	527.9

<sup>\*</sup> Clean-dry wool as percentage of raw (greasy) wool.

A marked difference in yield between the groups occurred at the end of the period, presumably much greater than would be expected from starch-contamination alone. It is therefore concluded that a greater elaboration of non-wool products had taken place in Group II, as well as a greater production of fibre.

The attempt was made to secure areas of similar size when they were first defined, although this was only possible within wide practical limits. It is unnecessary to give details of the raw sample-weights; in general they followed changes similar to those of the raw fleece weights, shown in Table II. The mean raw sample-weights were, before experiment, Group I, 5.72 gm.; Group II, 6.51 gm. with standard errors  $\pm 0.28$  and  $\pm 0.60$  respectively. After experimental feeding, Group I, 2.04 gm. with error  $\pm 1.12$  and Group II,  $4.28\pm0.47$ . Between the two groups there occurred a difference in the mean percentage changes in raw weight of 30.82, with standard error  $\pm 6.70$  (cf. Table II).

The clean-dry weights of the samples are given in Table IV. Whereas prior to experimental feeding there was an insignificant difference of 0.6 gm. between the mean dry weights, after the period of fee ling the difference was 1.1 gm., with standard error  $\pm 0.33$ ; and while the production fibre-substance in Group I, on the basal rations, changed 67.4 per cent. ( $\pm 3.27$ ) the mean amount grown on the defined areas of the sheep that were fed on the basal ration and starch, changed by 49.8 per cent. ( $\pm 4.30$ ). The significance of this difference in production confirms the above conclusion to the effect that the starch supplement definitely favour wool-growth.

Table IV.

Clean-dry weights of samples (in qm.)

	Gre	OUP I	:		Gro	UP II	
Animal	Pre- experi- mental	Experi- mental	Per cent. change	Animal	Pre- experi- mental	Experi- mental	Per cent. change
1 3 7 9 11 13 15 47 20 Mean S. E.	5·33 4·46 5·15 4·86 4·10 3·50 5·00 5·20 4·31 3·34 4·53 ±0·23	1·84 0·93 1·57 1·83 1·25 1·53 1·03 1·23 1·35 1·78 1·43 ±0·10	65·5 79·2 69·5 62·3 69·5 56·3 79·4 76·7 68·7 46·7 46·7 45·27	2 4 6 8 10 12 14 16 18 19	5.65 4.86 4.82 3.08 5.13 7.82 6.15 7.21 5.03 3.51 5.13 ±0.52	1·69 1·46 2·19 1·79 1·48 3·27 3·78 3·98 3·38 2·31 2·53 ±0·31	70·1 70·0 54·6 41·9 52·7 58·2 38·5 44·8 32·8 34·2 49·8

Differences: pre-experimental, 0.6  $\pm$  0.57. experimental, 1.1  $\pm$  0.33; percentage changes, 17.6  $\pm$  5.40.

(c) Fibre-length. In the determination of mean fibre-length, the method of zoning and measuring elaborated by Roberts [11] was used. The original samples were each divided into eight zones, and the proportions of fibres measured from the final sub-samples were 1 in 3 or 1 in 4, in the pre-experimental samples, and 1 in 8 or 1 in 10 in the samples taken at the end of the experimental period. The fibres were measured to the nearest half-centimetre.

In zoning and in counting out the fibres to be measured, great care was exercised to reduce as much as possible the chance of breaking fibres. In some samples, especially among those of Group I, taken after the feeding period, a considerable number of very short fibres (of less than half the mean fibre-length) were found. These caused the distribution of fibre-length in the particular samples to exhibit marked skewness, but their contribution to the total lengths of the fibre was small. (The greatest proportion was found in animal number 4, Group II, after experiment, where short fibres of 2 cm. or less, formed 34.9 per cent. of the fibres measured; their contribution to the total length was, however, only 6 per cent.) In measuring, the short fibres were treated as separate groups, and they are not included in the figures for mean fibre-length, shown in Table V. Since the total length enters into the determination of fineness, the short fibres are involved in the calculation of the results shown in the succeeding sections.

Table V.

Mean fibre-lengths (in cm.) excluding short fibres.

	Gra	oup I	×	Group II					
Animal	Pre-exptl.	Exptl.	Per cent.	Animal	Pre-exptl.	Exptl.	Per cent.		
3 5 7 9 11 13 15 17 20	9·2 10·4 10·8 9·3 8·9 8·2 8·1 9·5 8·3	5.2 6.3 6.4 5.9 5.1 5.2 5.7 6.0	-41.6 54.4 39.4 40.8 36.6 26.8 37.8 35.8 40.0 28.4	2 4 6 8 10 12 14 16 18	9·9 10·3 9·5 9·1 9·7 11·7 9·4 10·1 8·7 7·1	5·5 6·7 6·5 6·4 6·7 7·2 7·3 7·4 7·2 5·6	-44.4 35.0 31.6 29.7 30.9 38.5 22.3 26.7 17.2 21.1		
Mean	9-22	5.82	38.1		9.55	6.65	29.7		

Differences of means; pre-experimental, 0.33  $\pm 0.47$ ; experimental, 1.02  $\pm 0.30$ ; percentage changes, 8.4  $\pm 3.56$ .

In so far as fibre-lengths affect the appearance of staple-length in such wools, these figures illustrate one aspect of the difference between the groups. The change in production, considered from the standpoint of fibre-length alone, was marked in the basal-ration group, and the feeding of the starch supplement conferred a small but significant advantage in respect of growth in fibre-length.

If the short fibres are included, this distinction between the groups tends to be diminished and the effect of the different nutritional levels is reduced. The position in respect of mean fibre-lengths with short fibres included is summarized in Table VI.

Table VI.

Mean fibre-lengths, including short fibres.

the regulation of the second o	and the state of t			Group I	S. E.	Group II	S.E.
Pre-experimental .	٠.	•		8.98	±0.55	9*29	±0.40
	. 51				Θ.		
Experimental .	* *		• , •	5.30	±0.30	6-07	±0.26
Per cent. change, me	an .	•		41.0	±3·01	33·8	±3.42

This points to the conclusion that the difference in production on the sample areas was expressed more in other ways than in mere length of fibre grown, so that the other factors of average fibre-fineness, and, possibly, the number of fibres produced (i.e., number of active follicles) have to be taken into consideration.

(d) Average fineness. After counting and measuring the fibres, the sub-samples were washed in benzene and their clean-dry weights were determined. From the calculated total lengths of fibre and these weights, the values for fineness expressed as length (in cm.) per unit weight (mgm.) were derived.

Even under ordinary conditions, some change in fineness might be anticipated in all the animals, as the growth-periods cover two different stages in the maturation of the lamb's coat. The two groups at the outset were well balanced as regards fineness, and it will be seen that the different metabolic levels produced markedly dissimilar effects. In all animals, except No. 19 (Group II), the wool was distinctly finer at the end of the feeding period, and there is some general

262 THE INDIAN JOURNAL OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY [ IV, III similarity between the changes in individual fineness and those in individual body-weight (cf. Tables I and VII).

Table VII.

Average fineness.

_		Group I		X .	Gro	oup II quo	
Animal	Pre-exptl.	Exptl.	Per cent. change	Animal	Pre-exptl.	Exptl.	Per cent
1	112	167	+49-4	2	80	144	+61.8
3	132	206	56·1	4	114	140	22.8
5	104	159	52.9	. 6	94	120	27.7
7	116	163.2	41.0	. 8	105	112	6-7
9	118.5	191	61.2		98	117	19.4
11	88	124	40-9	12	113	144	27.4
13	113	175	54.9	14	119	144	21.0
15	143	281	96.5	16	125-5	146	16.3
17	97	159	63.9	18	114	127	11.4
20	117	132.6	13.3	19	162	161	-0.0
Mean	114.1	175.8	53.0		113.4	105.5	
S. E.	±5·02	土13.97	士6.64		±6·51	135·5 ±4·95	21·4 ±5·32

Differences of means: pre-experimental, 0.7; experimental, 40.3 ±14.82; percentage changes, 31.6 ±8.51.

These figures not only show responses of different magnitudes in the two groups, there being less alteration of fineness in the starch-supplement group. but also indicate a differential response as between length-growth and fineness. In Group I the reduction of growth conditions to the basal ration led to a difference in fibre-length production of 41 per cent. and a difference in fineness of 53 per cent.; whilst in Group II the effect of the starch supplement was to limit the orders of the reductions to 33.8 per cent. in length and 21.4 per cent. in fineness. That is the administration of the starch supplement gave a level of fibre-production which was nearer to that under pre experimental conditions, by affecting both fibre-length and fineness; but the proximity to the pre-experimental growth in Group II (as compared with Group I) was achieved more by encouraging the coarser (less fine) growth than by sustaining the fibre-length growth. In other words, the

fibres tended to respond to the starch supplement more in the direction of greater thickness ('diameter') than in greater length-growth.

(Note:—The values for fineness as expressed in cm./mgm. are inversely proportional to the squares of fibre 'diameters.') So far as the average fibre-growth is concerned, the situations are epitomized in the average fibre-volumes produced under the two nutritional levels.

(e) Average fibre-volume. The factors of mean fibre-length and average fineness can be combined to give measures of the volume of fibre-production. On the assumption that the density of the fibre-substance remains constant in wools of the same type, average fibre-volume is proportional to mean length upon average fineness. These values are shown in Table VIII.

Table VIII.

Average fibre-volume.

	****				Group I.	S. E.	Group II.	s. e.
Pre-experimental .	•			٠	0.080	±0.0048	0.085	±0.0059
Experimental .		•	•	•	0.032	±0.0034	0.045	±0.0028
Per cent. change, m	ean.		•	• ,	60.2	±3.53	44.3	±4.46

Since in Group I the change in mean length was of the order of 41 per cent., the remainder of the average change in fibre-volume, i.e., 19 per cent., must be attributed to change in 'diameter'; similarly in Group II, with length accounting for 34 per cent. the 'diameter' may be considered as contributing 10 per cent. to the average change in fibre-volume. Thus in the decrease of fire-growth due to the basal ration, the growth in respect of length contributed approximately twice as much as the change in thickness or 'diameter', whilst in the decrease on the pre-experimental condition of the starch-supplement group, the change in length was responsible for about three times as much as the change in 'diameter.'

The changes in the total production of clean wool on the defined areas (see Table IV) were of the order of 67 per cent. and 50 per cent. in Groups I and II, respectively, so that in each case there appears a margin beyond that attributable to alterations in average fibre-volume. The practical difficulties in recording with accuracy any actual change in the extent of a defined skin area on the living animal preclude the possibility of estimating contributions from such a source. Also differences in the amount of medullation present in the fibres affect the accuracy of the estimates of average fineness and fibre-volume.

(f) Estimated number of active follicles. The possibility of change in the number of follicles actively producing fibres on any area has been frequently

neglected in studies of wool-growth although such a phenomenon may conceivably occur according to age, season, or nutritional supply to the follicle, and has been demonstrated in the guinea-pig [12]. The observation that the starch-supplement group tended to exhibit denser and bulkier staples, which was confirmed when the samples of different wools from different areas were examined by an experienced wool buyer, emphasized the desirability of considering this factor. As with the other fleece characters, any difference between the two groups could reasonably be attributed to the effects of the ration.

The estimates of number of active follicles were made from the clean-dry weights of the sample and the zoned sub-sample, and the number of fibres included in the latter.

Table IX.

Estimated number of active follicles (00 omitted).

	Gro	T ADO			Gı	ROUP II	
Animal	Pre- experi- mental	Experi- mental	Per cent. change	Animal	Pre- experi- mental	Experi- mental	Per cent. change
1	652	558	-14:4	2	524	467	10.9
3	666	523	21.5	4	551	444	19.4
5	517	422	18.4	6	492	465	5.5
7	534	473	11.4	8	379	325	14.3
9	546	414	24.2	10	335	265	21.0
11	358	323	9.8	12	756	688	9.0
13	692	473.5	31.6	14	784	785	+0.1
15	955	779	18.4	16	908	851	-6.3
17	453	384.5	15.1	18	688	648	5.8
20	484	397	18.0	19	822	723	12.0
Mean	586	475	18.3		624	566	10.4
S. E	± 52	±40	± 2·02		±62	±63	±2.07

Differences of means: pre-experimental, 38  $\pm$  81; experimental, 91  $\pm$  75; percentage changes, 7.9  $\pm$  2.89.

Whatever may be the causes of the changes in the number of active follicles, as shown by these estimates, the numbers are apparently lower in the basal-ration group than in that receiving the starch supplement, although the differences are not significant. Regarded from the aspect of percentage changes in individuals, the alterations in Group I are greater than in Group II suggesting that this factor may have contributed to the margin in fleece-production over and above that assessable to the output of fibre-substance (volume) by the active follicles.

# CONCLUSIONS.

The advantage in respect of gross fleece-weight conferred by adding the starch supplement to the basal-ration is shown from the study of the defined sample areas to be largely due to the greater activity of the follicles in the elaboration of fibre-material. When the difference in fibre-production is expressed in terms of the average fibre-volume, the starch-fed group showed a decrease on their performance pirior to experiment of 44 per cent., as contrasted with a diminution of 60 per cent. in the basal-ration group. (This omits the distinction due to the different periods of time of pre-experimental and experimental growth.) The margin of difference between the order of such estimated change in fibre-volume and that of the amount of clean wool produced is possibly attributable to an alteration in the number of follicles which are actively producing fibres. In Group I, on the basal-ration, 18 per cent. fewer follicles were estimated to be operative, whilst in Group II the estimated diminution was 10 per cent.; in the former, those which were producing were doing so at the rate of 0.032 volume-units during the experimental period; in the latter their average volume-production was 0.045 units.

The mean length of fibre, all fibres included, did not differ significantly in the two groups, but if the shorter fibres (which conceivably contribute little to the apparent staple-length) are neglected, the average growth on the sample areas of the starch-fed group was about 1 cm. longer than that on the areas of the basal-ration group. The difference in rate of production due to the starch supplement was manifested more as a difference in the fineness of the fibres, in the sense that the fibres were maintained at a fineness nearer to their pre-experimental value than were those grown in the basal-ration group, the respective differences being, in length, Group I 41 per cent. and Group II 34 per cent., and in fineness 53 per cent. and 21 per cent.

#### DISCUSSION.

The addition of starch to the maintenance ration of young Cheviot sheep produced a significant increase in both fleece-weight and body-weight. The increase of fleece-weight was largely due to a definite increase in fibre-thickness, a slight

increase in fibre-length, and possibly also to an increase in the proportion of follicles setively elaborating fibres. Additional carbohydrate in the diet therefore led to the increased production of a substance predominantly protein in nature.

The depression of wool-production in the basal group might presumably be due to one of two causes:—

- (a) The protein in the diet might have been used mainly for supplying energy. The influence of starch in increasing wool-production would then be an illustration of the well-known protein-sparing action of carbohydrates.
- (b) The activity of the wool follicles might have been depressed by the low energy-content of the basal diet. The addition of the starch would then have increased the metabolic activity of the follicles, leading to increased rate of elaboration of their characteristic product.

The theoretical question at issue is whether the depression of wool-production in the basal group was due to decreased activity of the wool-folliele, or to a deficiency of the substances required for the material expression of that activity. It is possible that both factors were involved.

The results of the experiment have an important practical application to pastoral conditions. Owing to the protein nature of wool, it has been widely assumed that reduced wool-production must be associated with deficiency of protein in the pasture. Yet under drought or other famine conditions where sheep are losing weight, it is evident that any protein supplement fed will be used in the first place as a source of energy. It is therefore obviously equally efficacious, and usually more economic, to feed the cheapest available carbohydrate supplement of high caloric value. Such a supplement, in the light of the experiment here recorded, may be expected not only to maintain normal body-weight, but normal wool-production as well.

#### SUMMARY.

- (1) One group of ten growing sheep was fed on a maintenance ration. A second group of ten was fed on the same ration supplemented with maize starch.
- (2) The starch produced a significant increase in both body-weight and gross fleece-weight.
- (3) The increase in gross fleece-weight was reflected in the weight of clean wool.
- (4) This increase was due to a definite increase in fibre-thickness, a slight increase in fibre-length, and possibly also to an increase in the proportion of follicles actively elaborating fibres.

(5) Theoretical interpretations and the practical applications of the experimental results are discussed.

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# ON THE INCIDENCE OF STOMACH WORMS IN LAMBS IN THE NORTH OF SCOTLAND AND THEIR CONTROL BY PROGRES-SIVE SECTIONAL GRAZING.

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(Reprinted from the Journal of Helminthology, Vol. XI, No. 4, October 1933)

# INTRODUCTION.

Recent investigations carried out by the senior writer on the incidence of parasitic helminths in the alimentary tract of sheep in the north of Scotland have demonstrated heavy losses in lambs due to parasitic gastritis. In the autumn of 1932 six severe outbreaks were recorded in the counties of Kincardine, Aberdeen and Moray, but no doubt this number would have been greatly exceeded had it been possible to make a comprehensive survey. Some idea of the severity of the disease may be judged by the fact that several farmers lost from 10-20 per cent. of their lambs.

The usual predisposing cause was observed in each case, namely, over-stocking attended by failure to move the lambs on to fresh ground when symptoms of parasitic infestation were apparent.

Postmortem examination of diseased lambs revealed that the lesser stomach worm, Ostertagia circumcineta was present in very much larger numbers than any other parasite. O. trifurcata sometimes occurred in small numbers but no attempt was made to separate the two species. The Table below gives the numbers and species of helminths found in two typical cases of parasitic gastritis.

A further 6 untreated lambs from different areas were autopsied but no record of the actual numbers of worms present was taken. A close examination of the abomasum contents was made, however, and in each case the number of O. circumcincta far exceeded that of H. contortus or any of the intestinal worms found present. It may, therefore, be concluded that parasitic gastritis in lambs in the north of Scotland is mainly due to O. circumcineta and that any control measures

directed with a view to checking the disease should be carried out against this parasite.

TABLE I.

Showing the number and species of helminths found in the intestinal tract of 2 lambs suffering from parasitic gastritis.

				-	Nu	MBER
Organ	Species				Morayshire	Kineardinoshire
					Killed 26th October 1932	Killed 16th September 1932
Abomasum	Ostertagia circumcincta . Hæmonchus contortus .		•	• 1	16,930 53	18,110
Small intestine .	Nematodirus filicollis Trichostrongylus vitrinus Cooperia curticei Monodontus trigonocephalus	:	:		1,238 256 98 7	1,981 162 354
Large intestine .	Chabertiz ovina Oesophagostomum venulosum	<i>e</i> •			19 8	26
Cæoum	Trichuris ovis	•			8	12

The fact that in Scotland *H. contortus* occurs in sheep in relatively small numbers compared with *O. circumcincta* is not without interest. Generally speaking in U. S. A. and South Africa the reverse is the case, *H. contortus* being more important from an economic point of view than *O. circumcincta*, although according to a recent report on parasitic diseases in U. S. A. (1932) the lesser stomach worm is stated to be more common in some parts than others, particularly on the West Coast. A similar state of affairs exists in Australia, for according to Seddon and Ross (1929), *H. contortus* is of the greatest importance as the cause of parasitic gastritis in New South Wales and Queensland, but to a less extent in the Southern Australian States where infestation with *O. circumcincta* appears to be more common.

The literature on the control of stomach worms by the administration of anthelmintics is extensive and it is well known that *H. contortus* can be readily controlled by dosing with copper sulphate. Unfortunately *O. circumcineta* is much less susceptible to the action of anthelmintics and no drug which will remove this parasite effectively from the abomasum has as yet been discovered.

Control of stomach worms by a system of folding pastures has long been advocated, and some of the results obtained by this method for *H. contortus* have been

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Control of stomach worms by a system of folding pastures has long been advoeated, and some of the results obtained by this method for *H. contortus* have been highly successful. Such a system of control is based on the nature of the life cycle of the parasite, which has been worked out in detail by Veglia (1916). There does not appear to be any complete account of the life cycle of O. circumcincta, but most workers are of the opinion that it follows the same course as H. contortus in which the succession of events is as follows:—

The eggs laid by the mature females in the abomasum reach the ground in the fæces and the larvæ hatch out in about 24 hours. These feed on certain bacteria in the fæces and must pass the next two stages of their development on the ground before they are capable of infecting the sheep. The time taken to reach the infective stage may vary from 3 days to several weeks, depending on the temperature and degree of moisture present.

It can be readily understood that the time taken for the newly hatched larvæ to reach the infective stage is the all important factor in regard to the introduction of any system of control by progressive sectional grazing of the pastures. In this connection Taylor (1929) states for both H. contortus and O. circumcincta that, 'although in this country development would rarely be delayed because of excessive dryness, the temperature is usually below that best suited to the larvæ and it can only be in our warmest weather that they reach the infective stage in less than 10 days'.

Assuming that the larvæ of O. circumcincla require a period of ten days to become infective it follows that any system of progressive sectional grazing whereby the lambs are shifted on to clean pasture every 10 days should give complete control. Before advising control measures along these lines, it was decided to carry out a preliminary experiment with a view to gaining information on the following points:—

- (1) The extent to which stomach worm infestation of lambs can be reduced by a system of progressive sectional grazing.
- (2) Whether 10 days is a safe maximum period to allow between successive shifts in reducing infestation from O. circumcincta.
- (3) The degree to which lambs can become infected when put to graze with their infested dams on clean pasture.

The experiment was undertaken jointly under the north of Scotland College of Agriculture and the Rowett Research Institute, the latter providing all facilities for the investigation.

# PLAN OF THE EXPERIMENT.

A three acre field of excellent third year's grass which had never carried sheep was fenced into equal portions. Both portions were on a gradual slope and comparable in every respect as regards grazing and drainage.

Six Blackface ewes and their twelve twin Greyface lambs approximately six weeks old were put into one half of the field on 25th May 1932, and allowed to graze over the whole 13 acres up to the 28th August 1932.

Table II.

Showing the number of eggs per gram of faces from lambs at 6 weeks old.

P.=Progressional group	r	N. P.= Non-progressional group	group	
P. 1=85		+	N. P. 1=75	1
P. 2==66			N. P. 2=52	
P. 3=66			N. P. 3=81	

An equal number of ewes and lambs were allowed to graze only 1-10th of the other half at one time, the ewes and their lambs being changed to clean sections at 10-day intervals. A temporary fence of sheep stakes and wire-netting served to confine the animals.

Both groups of lambs were selected as nearly alike as possible with respect to age, weight and condition. The average weights of the progressional and non-progressional groups were 23.4 lbs. and 23.9 lbs. respectively.

Prior to the commencement of the experiment the ewes and lambs were submitted to fæcal examination for the presence of stomach worms. Fæcal cultures demonstrated the presence of a moderate to heavy infestation of both *H. contortus* and *O. circumcincta* in all the ewes.

Three lambs from each group were submitted to egg counts by the Stoll method (Table II). The fæces were removed by hand, the method being to insert the small finger into the rectum.

Since it is not possible from an examination of eggs to determine the species of worms present, fæcal cultures were made. Approximately 90 per cent. of the migrating larvæ were identified to be O. circumcincta, the remaining 10 per cent. being Nematodirus and Trichostrongylus species. A lamb from the same source and similar in age and weight to those submitted to fæcal examination was autopsied and examined for helminths. The abomasum yielded 76 O. circumcincta but no H. contortus, while the small intestine colon and execum contained no helminths. It may be concluded therefore that both groups of lambs commenced the experiment with an initial infection of helminths consisting almost entirely of O. circumcincta.

# PROGRESS OF THE EXPERIMENT.

Owing to the scarcity of grass at the commencement of the experiment it was found necessary to change the progressional group to the second section after a period of 5 days. Each subsequent section was grazed for an interval of 10 days. Towards the first week of June the grass came away quickly, in fact too quickly as the rank nature of the grass in the ungrazed sections led to much trampling and wastage of the grass, so much so, that by the end of the 10-day interval the lambs were not finding sufficient for their requirements. This meant that the lambs in the progressional group were getting less food than the non-progressional group and could, therefore, not be expected to put on the same weight. The difficulty could have been met by increasing the grazing area of the progressional group, but since the main object of the experiment was to find out to what extent sectional grazing would lessen the degree of infestation to stomach worms, it was considered inadvisable to take this step. From a nutritional point of view then, the non-progressional group had a considerable advantage over the progressional group, as the grass in their plot was plentiful to the end of the experiment.

On the 28th August the lambs were weighed and then removed to a local abattoir for slaughter (Table III). The abomasums were removed and the worms were isolated by the usual process of sedimentation and decantation and counted (Table IV).

TABLE III.

Showing the weight of the lambs at beginning and the end of the experiment.

Progressi	onal group=P.		Non-progressional group=N. P.				
	Date of	weighing		Date of	weighing		
	25th May, 1932	28th Aug., 1932		25th May, 1932	28th Aug., 1932		
P. 1	21·5 21 22·5 27 20 23·5	57 52 54 55 48 57	N. P. 1	24 23.5 21.5 21 25.5 28	61 64 67 67 68 73		
				P. Group	N. P. Group lbs.		
Average weight of th Average weight of lar	e lambs at the nbs at the end	beginning of t the experimen	he experiment t	23·4 53·8	23·9 66·6		

TABLE IV.

Showing the total numbers of worms recovered from the abomasums of the progressional and non-progressional groups.

Progressional group=P.				Non	-pro	gressi	sional group=N. P.			
	\$***************************			H. contor- tus	O. circum- cineta		-		H. contor- tus	O. circum cincta
P. 1				1	1	N. P. 1 .			452	1,177
P. 2				. 0	36	N. P. 2 .			349	1,132 -
· 3	•	• ,		1	384	N. P. 3 .			233	927
2. 4	•		•	2	437	N. P. 4.			363	1,023
. 5				8	132	N. P. 5 .			86	690
. 6				0	6	N. P. 6 .			61	246

# DISCUSSION OF RESULTS.

In the case of *H. contortus* the total number of worms recovered from the six lambs of the non-progressional group was 1,550, or an average of 258 per lamb. From the progressional group only 12 worms were recovered, giving an average of 2 per lamb. These results show that progressional grazing was efficient to the extent of reducing *H. contortus* infestation 99.2 per cent. compared with the non-progressional group. In other words *H. contortus* can be completely controlled by this system.

The average number of *O. circumcincta* recovered from the non-progressional and progressional groups was 865 and 166 respectively, giving an efficiency of 80.8 per cent. The lower percentage of reduction in the case of *O. circumcincta* would point to the fact that some larvæ were able to reach the infective stage in less than 10 days. For all practical purposes, however, 10 days may be considered a safe period for this species, as the few larvæ that may develop to the infective stage within that time are not likely to cause much harm.

Statistical analysis of these results kindly undertaken by Dr. Tocher, Aberdeen, indicate that the odds against the results being fortuitous are 700 to 1, and 1,000 to 1 for *H. contortus* and *O. circumcineta* respectively.

The number of worms recovered from the non-progressional group indicate the degree to which lambs may become infested when put to graze on worm-free

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pasture with their infected dams. Although the numbers are too small to cause serious harm, the evidence shows the advisability of dosing ewes and having them as free of worms as possible before putting them to graze with their lambs on new grass.

Acknowledgments. We wish to thank Mr. E. L. Taylor, Veterinary Laboratory, Weybridge, Professor James Ritchie, Natural History Department, Aberdeen University, and Dr. Orr, Director, Rowett Research Institute, for their helpful criticism.

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# PYRETHRUM AS AN ANTHELMINTIC FOR ASCARIDIA LINEATA.

#### BY

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Over a century ago, it was discovered that flowers from certain species of the pyrethrum plant would kill various forms of insect life. The powdered flowers of this plant have been exported under various names from certain provinces of Persia and Dalmatia into European countries for use as insecticides. The drug was introduced into America first about the year 1850.

The pyrethrum plant belongs to the genus Chrysanthemum, of the family Compositae. Ginsburg¹ lists the following species as being toxic for insects: (1) roseum, (2) carneum and (3) cinerariaefolium. The last-named species is cultivated especially for insecticidal purposes because it is higher in insecticidal properties and gives a larger yield of flowers.

Pyrethrum is very poisonous to insects and cold-blooded animals, but it is not toxic to man or other warm-blooded animals. Although all parts of the plant are toxic to insects, most of the toxic principle is found in the flower-head. The toxicity is due to two chemical compounds named pyrethrin I ( $C_{22}H_{30}O_5$ ). These compounds are highly insoluble in water but dissolve readily in most organic solvents such as alcohol, acetone, ether and kerosene. The total pyrethrin content of the dried flowers varies from 0.3 to 1.2 per cent. The term pyrethrum refers to the entire plant, while pyrethrin designates only the active principle. Within recent years, pyrethrum has become one of our most valuable insecticides and large quantities of the flowers are imported annually into this country from Japan, Italy and other countries where this plant is cultivated.

Staundiger and Ruzicka<sup>2</sup> described the active principles of pyrethrum and the toxic action on cold-blooded animals, and stated that oral administration to warmblooded animals was without danger.

Chevalier<sup>3</sup> began internal medication with this drug in man and domestic animals. He found it to be very efficacious in removing ascarids and tenia from dogs, and stated that young dogs would withstand large doses of this drug without apparent discomfort. A dose of 10 mg. was found to be sufficient to remove all tenia and ascarids from the dog.

<sup>\*</sup> In co-operation with the Ohio Agric. Expt. Stn., Animal Disease Laboratories, Raynoldsburg, Ohio.

Urbain and Guillot,<sup>4</sup> of the French Army Veterinary Service, reported the results of their work on the administration of pyrethrin for the removal of intestinal parasites of horses. Ascarids, oxyurids and strongylids were removed with a formula consisting of 1 gm. of the drug in 20 gm. of easter oil. These investigators state that the superiority of pyrethrin lies in its definite lethal rather than paralyzing effect upon the parasites, and that the use of laxatives is unnecessary following its administration.

The satisfactory results obtained with pyrethrum in mammals suggested the possibility that this preparation might be efficient in removing intestinal parasites of poultry. A series of tests were conducted to determine the anthelmintic value of pyrethrum for Ascaridia lineata.

# METHODS.

Thirty adult birds, known to be infected with Ascaridia lineata, were placed in individual cages having wire-mesh bottoms and removeable pans for the droppings. Powdered pyrethrum in gelatin capsules was administered to each bird after feed had been withheld for a period of 12 hours. The droppings were collected in water for a period of 72 hours and examined for the presence of ascarids. Post-slaughter examinations were made at the end of five days following treatment; the intestines were opened and their contents scraped into a vessel containing tap water. Both the scraped intestine and the diluted contents were examined for the presence of Ascaridia lineata.

Since no record could be found of the use of pyrethrum for the removal of intestinal parasites in the chicken, an arbitrary dose had to be determined. Based on the work of Chevalier in the dog and cat, it was decided to use 200 mgs. of the powdered pyrethrum.

Since the pyrethrin content of the dried floweres varies from 0.3 to 1.2 per cent., it was deemed advisable to use a preparation which contained not less than 0.7 per cent. The assay of the drug used in these tests showed a pyrethrin content of 0.8 per cent.

# EXPERIMENTAL DATA.

The result obtained by the administration of 200 mgs. of powdered pyrethrum (containing 0.8 per cent. pyrethrin) for the removal of Ascaridia lineata are given in Table I. This shows, for each host, the efficacy of the drug against Ascaridia lineata as indicated by the percentage of worms removed compared with the total number present.

Table I.

Anthelmintic value of pyrethrum for Ascaridia lineata (200 mg. pyrethrum containing

0.8 per cent. pyrethrin).

Bird	Ascaridia o	bserved in dro	ppings after t	reatment	Post- slaughter	Efficiency
	12 Hrs.	24 Hrs.	72 Hrs.	Total	observation	per cent.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25	8 16 9 1 10 8 0 0 0 4 15 12 8 0 0 0 0 0 4 15 12 8 0 0 0 0 0 0 0 0 0 0 0 0 0	6 2 4 0 2 4 8 2 6 10 5 0 0 0 8 3 2 0 0 1 4 5 6 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	2 0 3 0 0 0 0 4 0 4 6 6 0 0 0 0 4 3 3 8 5 3 1 0 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	16 7 25 13 1 12 12 12 12 16 15 15 15 12 8 12 15 3 8 14 9 16 15	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	100 100 100 100 100 100 100 100 100 100
26 27 28 29 30	4 0 3 6 0	2 0 6 4 8	4 0 2 8 2	10 0 11 18 10	0 4 0 0 1	100 0 100 100 90.9
Totals .	132	108	93	333	17	95·14

In 24 of the 30 birds treated, complete elimination was obtained and only one bird failed to pass worms following treatment. Five birds passed some worms following treatment but did not eliminate all of them.

Birds 15 and 30 passed all worms except one and in each instance the remaining worm was found to be dead when the bird was slaughtered. Birds 19 and 20 passed the majority of worms present but did not have complete elimination. Bird 9 passed only 28.5 per cent. of the worms present and bird 27 failed to pass any. It is possible, in the latter case, that the capsule containing the drug may

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have failed to pass into the crop and was coughed out, although no trace of pyrethrum could be found in the dropping-pan.

The efficacy of pyrethrum against Ascaridia lineata, based on the worm total, was found to be 95°14 per cent. A total of 333 Ascaridia lineata were moved from the 30 birds treated and 17 were found at post-slaughter examination. The largest number of worms were removed in the first twelve hours following treatment, 132 being recovered. At the end of 24 hours, an additional 108 worms were recovered and 93 were passed between the 24th and 72nd hours.

The foregoing data indicate that pyrethrum (containing 0.8 per cent. pyrethrin) has a high efficiency in removing *Ascaridia lineata* from the chicken. It has an added advantage in that no purgative is required.

It is quite possible that pyrethrum may be found to be highly efficient against other species of parasites. Tests are being conducted to determine its efficiency against tapeworms infecting the chicken.

# SUMMARY.

Powdered pyrethrum in doses of 200 miligrams, containing 0.8 per cent. pyrethrin, was administered to 30 adult chickens, to determine the efficiency of the drug in removing Ascaridia lineata. The droppings of each bird, voided over a period of 72 hours following treatment, were examined for Ascaridia lineata. These birds were then killed and examined for the presence of Ascaridia lineata. Twenty-four birds eliminated all Ascaridia lineata following treatment and only one bird failed to pass worms. Five birds passed Ascaridia lineata but did not completely eliminate them. The efficacy of pyrethrum against Ascaridia lineata, based on a worm total, was found to be 95.14 per cent.

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# CANINE RABIES EXPERIMENTAL VACCINATION\* SECOND AND THIRD REPORTS.

 $\mathbf{B}\mathbf{Y}$ 

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This third report is designated 'Second and Third Reports' because it involves and supplements the second report which was presented at the Veterinary Conference in Philadelphia, in January, 1932, but which was not published.

Our first report contained a description of the purpose of our experiments, the location and description of the grounds and buildings, the sources of the dogs used and susceptibility tests, and a preliminary report of the results of the use of carbolized canine rabies single dose vaccines obtained from four different commercial laboratories. These four vaccines, in order not to disclose the identities of the laboratories from which they were obtained, were designated as vaccines A, B, C, and D.

The first report contained the following summary:

Of 27 dogs vaccinated and later exposed 24 (89 per cent.) died of rabies.

TABLE I.
Summary of results of the use of carbolized vaccine.

1-10		, ii		Died		
Tre	atment	Dogs	Rabies	Rabies not proved	Other causes	Alive.
Vaccine A		10	7	2	1	0
Vaccine B		10	- 9	0	0	1
Vaccine C		10	8	0	1	1
Vaccine L		10	9	0 -	0	1
	Totals .	40	33 (82.5%)	2	2	3
	Controls .	16	13 (81.25%)	1	1	1

<sup>\*</sup> Presented at the seventieth annual meeting of the American Veterinary Medical Association, Chicago, Ill., August 14-18th, 1933.

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Of 11 unvaccinated controls exposed, 10 (91 per cent.) died of rabies.

Since the report was made, the remainder of 40 vaccinated dogs and additional controls were exposed, and the final summary of the results of dogs treated with carbolized vaccine is presented in Table I.

#### DISCUSSION.

The results of our studies, in the use of the single-dose carbolized canine rabies vaccine, indicated that none of the vaccines used were capable of immunizing dogs against any of the viruses used.

There was some indication that the injection of street virus did not immunize dogs against a subsequent injection, 60 or more days later, of either the same strain or a different strain of virus.

The single-injection method of vaccinating dogs with carbolized vaccine is apparently unreliable and does not immunize dogs against rabies, and, therefore, should not be relied upon as a means of controlling rabies in dogs.

#### CHLOROFORM-TREATED VACCINE.

After the first experiments in the use of carbolized vaccines were nearing completion, another group of healthy dogs was inoculated by different channels—subarachnoid intramuscular and intravenous—with various dilutions and various-sized doses of rabies virus to determine, if possible, the comparative minimum fatal dose and to form a better basis for exposing dogs after vaccination with a chloroform treated vaccine. In these tests, only one virus (27039) was used and dilutions were made so that 0.5 c.c. represented the dose in each case, Emulsions ranging from 10.0 per cent. down to 0.009 per cent. were used, and the 0.5 c.c. dose contained amounts of brain material ranging from 0.8 grains (50 mg.) down to approximately 0.0007 grains (0.05 mg.). The results were somewhat irregular but there seemed to be some indication, within certain limits, that the dispersion of virus by dilution, rather than the amount of virus, had some relation to infections which took place.

On August 20, 1931, 20 healthy dogs were vaccinated with commercial (Laboratory A) chloroform-treated rabies vaccine prepared by the Kelser method. An equal number of dogs were set aside as controls. These groups were sub-divided and, later, individual dogs or groups were exposed through different channels to different strains of virus as is shown in the accompanying tables.

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TABLE IV.

Pasteur virus 1850EE5 (10 per cent. cmulsion).

			* - * -			
	Dog Vaccinated	Date	Method	Dose (c.c.)	Results	
248		1	A STATE OF THE STA	Subarachnoid .	0.1	R. * 9 days.
228	• •	20-8-31		Subarachnoid .	0.2	R. * 9 days.
174	. 1.	J	17-11-31	Intravenous	0.5	Lived.
290	· · ·	)	17-11-51	(Subarachnoid .	0.1	R. * 8 days.
353		Controls	••	Subarachnoid .	0.2	R. * 8 days.
391			-	Intravenous .	0.2	Lived.

<sup>\*</sup> Rabies.

TABLE V.

Street virus 27039B71 (10 per cent. emulsion).

Dog	Vaccinated	* * *	Virus exposure				
Dog	Vaccinated	Date	Method	Dose (c.c.)	Results		
84	1		(Subarachnoid .	0.1	R. * 12 days.		
	20-8-31		Subarachnoid .	0.2	R. * 15 days.		
31	20-8-31		Intravenous .	0.5	Lived.		
20	J	17-11-31	Intramuscular .	1.0	Lived.		
	7 4 4	11-11-31	(Subarachnoid .	0.1	P. * 16 days.		
1	Controls		Subarachnoid .	0.2	R. * 14 days.		
	Controls		Intravenous	0.5	Lived.		
07			Intramuscular .	1.0	R. * 52 days.		

<sup>\*</sup> Rabies.

TABLE VI.

Street virus 50012D223 and 214 (10 per cent. emulsion).

	Dog	Vaccinated		Virus exposure				
	Dog vaccinated		Date	Method	Dose (c.c.)	Results		
185 362 285 284		} 20-8-31	 17-12-31	Subarachnoid . Subarachnoid . Subarachnoid . Subarachnoid .	0·1 0·2 0·1 0·2	R. * 19 days. Lived. R. * 21 days. R. * 16 days.		

<sup>\*</sup> Rabies.

TABLE VII.

Street virus 27039B82 (1 per cent. emulsion).

	Dog	Vancinated		Virus exposure				
,	Dog Vaccinated		Date	Method	Dose (c.c.)	Results		
292 364 192 361		20-8-31	15-4-32	Intramuscular . Intramuscular . Intramuscular . (Intramuscular .	2·0 4·0 4·0	Lived. Lived. Lived. Lived.		
377 294	: :	Controls		Intramuscular .	4·0 4·0	Lived.		

TABLE VIII.

Summary of results of initial exposures (Tables II to VII inclusive).

Dogg	Vacci	nated	Controls
Dogs	20	*.	20
Died of rabies	No.	7	11
	%	35	55
Died (rabies not proved)	No.	0	1
	%	0	- 5
Lived	No.	13	- 8
	. %	65	40

INCUBATION PERIODS FOLLOWING ARTIFICIAL EXPOSURES.

The average period of time from exposure until death in 117 proved cases of rabies in dogs was 21.5 days. The usual period was between 13 and 25 days.

In 13 dogs (Table XII) showing a period of 30 days or more, the average was 59.8 days.

One dog showed a period of 223 days.

Twelve dogs showed an average of 46.3 days.

In 104 dogs showing a period of less than 30 days, the average was 16.7 days. Four of these were exposed to fixed virus and died in an average of 8.5 days.

In 100 dogs showing a period of less than 20 days, and which were exposed to street virus, the average period until death was 17 days.

#### Discussion.

There undoubtedly has been a greater increase in the number of centers of infection throughout the country, and the number of cases of rabies both in animals and in humans, since the single-dose rabies vaccine came into use, than for any other equal period of time known; yet the States are better equipped with transmissible disease control organizations for combating animal diseases. This increase may be partly due to a feeling of false security built up through the use of the single-injection vaccine.

We are convinced that the use of the canine rabies vaccines now on the market does not offer a successful means for the control and suppression of rabies.

If sanitary officials are going to cope successfully with the rabies situation to prevent an increase in the number of centers of infection, they must apply more strict sanitary police measures.

Table IX.

Results of subsequent exposures of survivors of previous experiments.

		VACCINAT	ED 20TH A	roust 19	931	
Dog	Date	Virus*	Emul- sion (%)	Dose (c. c.)	Method†	Result
242	16th November 1931	50012	10.	0.1	SA	
	21st July 1932 .	57382	1	0.2	SA	
	14th October 1932 .	58982	10	0.2	SA	
	26th June 1933 .	58982	10	0.25	SA	Alive 7th August 1933.
452	16th November 1931	50012	10	0.5	SA	
	21st July 1932 .	57384	1	0.2	SA	
	14th October 1932 .	58982	10	0.2	SA	
	26th June 1933	58982	10	0.25	SA	Alive 7th August 1933.
195	16th November 1931	50012	10	0.2	IM	
	15th April 1932 .	B82	1	2.0	IM	
	20th July 1932 .	B88	1 1	0.5	SA	
	14th October 1932 .	58982	10	0.5	SA	D. (?) § 15th November 1932.
318	16th November 1931	50012	10	0.3	IM	
	15th April 1932 .	B32	1 1	2.0	IM	7.00
	20th July 1932	B88	Ī	0.2	SA.	-, -
	14th October 1932 .	58982	10	0.2	SA	
	26th June 1933 .	58982	10	0.25	SA	Alive 7th August 1933.
453	16th November 1931	50012	10	1.0	IM	
. /	15th April 1932	B82	1 1	2.0	IM	
	20th July 1932	B88	1	0.2	SA	
	14th October 1932 .	58982	10	0.2	SA	
	26th June 1933 .	58932	10	0.25	SA	Alive 7th August 1933.
	I I		CONTROLS			

Dog	Date	Virus	Emulsion (%)	Dose (c. c.)	Method	Result
223	16th November 1931	50012	10	0.1	SA	R. ‡ 22 days.
214	16th November 1931	50012	10	0.2	SA	R. ‡ 21 days.
297	16th November 1931	50012	10	0.2	IM	D. (?) § 10 days.
258	16th November 1931	50012	10	0.9	IM	
	15th April 1932 .	B82	1	2.0	IM	
	20th July 1932 .	B88	1	0.3	SA	
	14th October 1932 .	58982	.10	0.2	SA	
	26th June 1933 .	58982	10	0.25	SA	Alive 7th August 1933.
436	16th November 1931	50012	10	1.0	IM	
	15th April 1932	B82	1	2.0	IM	
	20th July 1932 .	B88	1	0.2	SA	
14	14th October 1932	58982	10	0.2	SA	
4 1 19	26th June 1933	58982	10	0.25	SA	Alive 7th August 1933.

Note.—Previous history of dogs 242, 452, 195, 318, 453, 223, 214, 297, 258, and 436 given in table II.

Table IX.

Results of subsequent exposures of survivors of previous experiments—continued.

		VACCINATE	D 20тн A	gust 19	31	
Dog	Date	Virus*	Emul- sion (%)	Dose (c. c.)	Method†	Result
237	16th November 1931	51371	- 10	0.5	IV	* )
237	15th April 1932 .	51371R	2.5	1.0	IV	\
	21st July 1932	57384	1	0.2	SA	
	14th October 1932 .	58982	- 10	0.2	SA	
	26th June 1933 .	58982	10	0.25	SA	Alive 7th August 1933.
174	17th November 1931	1850EE5 (Pasteur)	10	0.5	IV	*
	15th April 1932 .	1850EE5	10	- 00		ś.
	Took April 1002 V	(Pasteur)	2.5	1.0	IV	
- 3	21st July 1932 .	57384	1	0.2	SA	
	14th October 1932 .	58983	10	0.2	SA	
	26th June 1933 .	67074	10	0.25	SA	Alive 7th August 1933.
1000	17th November 1931	B71	10	0.5	IV	- Alle Control of the Alle Control
381		B82	10	1.0	IV	
	15th April 1932				ŠĀ	
	20th July 1932	B88	1	0.2	SA	1 14
	14th October 1932 .	58982	10	0.5		71 4 10 1
	26th June 1933	67074	10	0.25	SA	Et. ‡ 13 days.
420	17th November 1931	B71	10	1.0	IM	
	15th April 1932 .	B82	1	2.0	IM	*
	20th July 1932 .	B88	1 1	0.2	SA	
	14th October 1932 .	58982	10	0.2	SA	R. ‡ 16 days.
Dog	Date	Virus	Emul- sion (%)	Dose (c. c.)	Method	Result
			STOIL ( 70)	(0. 0.)	1	
277	16th November 1931	51371	10	0.5	IV	
200	15th April 1932 .	51371R	2.5	1.0	ÎV	
" - E 2	21st July 1932	57384	1	0.2	SA	
A la	14th October 1932	58982		0.2	SA	D. (?) § 14th December
	1400 000000 1882 .	90002	10	0.2		1932.
391	17th November 1931	1850EE5			L. X	1.000.
281	1.00 10 (00000001 1001	(Pasteur)	10	0.8	IV	
	15th April 1932 .	1850EE5	10	0.5	TA	**
	Tom April 1992 .		9.8	7.0	777	= 1"
	21st July 1932 .	(Pasteur)	2.5	1.0	IV	
		57384	1	0.2	SA	
	14th October 1932	58982	10	0.2	SA	And Mark A
	26th June 1933 .	67074	10	0.25	SA	Alive 7th August 1933.
331	17th November 1931	B71	10	0.8	777	4
	15th April 1932 .		10	0.5	IV	200
	20th July 1932	B92	1	1.0	IV	
		B88	1	0.2	SA	X1 (0.5
	14th October 1932	58982	10	0.2	SA	
	26th June 1933	67074	10	0.25	SA	Alive 7th August 1933.
307	17th November 1931	B71	10	1.0	IM	R. 1 52 days.

Norm.—Previous history of dogs 237 and 277 given in table III; of dogs 174 and 391 in table IV; of dogs 381, 420, 331 and 307 in table V.

TABLE IX.

Results of subsequent exposures of survivors of previous experiments—concluded.

Dog	Date	Virus*	Emul- sion (%)	Dose (c. c.)	Method†	Result
362	17th December 1931 21st July 1932 14th October 1932	50012 57384 58982	10 1 10	0·2 0·2 0·2	SA SA SA	
	26th June 1933 .	67074	10	0.5	SA	Alive 7th August 1933.
292	15th April 1932 . 20th July 1932 .	B82 B88	1	2·0 0·2	IM SA	
	14th October 1932 . 26th June 1933 .	58932 67074	10 10	$\begin{array}{c} 0.2 \\ 0.25 \end{array}$	SA SA	Alive 7th August 1933.
364	15th April 1932 . 20th July 1932 . 14th October 1932 .	B82 B88 58982	1 1 10	4·0 - 0·2 - 0·2	IM SA SA	R. ‡ 17 days.
192	15th April 1932 20th July 1932 14th October 1932 26th June 1933	B82 B83 58982 67074	1 1 10 10	4·0 0·2 0·2 0·25	IM SA SA SA	Alive 7th August 1933.

CONT	ROLS
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	Dog	Date	Virus	Emul- sion (%)	Dose (e. c.)	Method	Result
	284	17th December 1931	50012	10	0.2	SA	R. ‡ 16 days.
	361	15th April 1932 20th July 1932	B82 B88	1	2·0 0·2	IM SA	- * *
		2nd November 1932.	58082	10	0.3	SA	D. (?) § 24th November 1932.
	377	13th April 1932 20th July 1932 2nd November 1932	B82 B88 58982	1 1 10	4·0 0·2 0·2	IM SA SA	D. (?) § 16th November
MAN AND AND AND AND AND AND AND AND AND A	294	15th April 1932 20th July 1932	B82	1	4.0	IM	1932. D. during inoculation.

Notr.—Previous history of dogs 362 and 284 given in table VI; of dogs 292, 364, 192, 361, 377 and 234 in table VII.

<sup>\*</sup> B71, B82, etc. =71st or 82nd passage through rabbits.

 $<sup>\</sup>dagger SA = Subarachnoid; IM = Intramuscular; IV = Intravenous.$ 

<sup>‡</sup> Rabies.

<sup>§ (?) =</sup> Rabies not proved.

TABLE X.

(Table IX condensed). Results of subsequent exposures of survivors of previous experiments.

	Vac	cinated	20th August 1	931.						
		E	cposures	· · · · · · ·			E	xposures	75 11 "	
Dog	Table	Num- ber	Last	Result	Dog	Table	Num- ber	Last	Result	
242	II	4	26th June 1933.	Alive 7th August 1932.	223	11	1	16th November 1931.	R.* 22 days.	
452	п	4	26th June 1933.	Alive 7th August 1932.	214	11	1	16th November 1931.	R.* 21 days.	
195	II	4	14th October 1932.	15th Novem-	297	II .	1	16th November 1931.	D. (?); 20th No- yember	
		1.0		ber 1932.		1			1931.	
318	II	5	26th June 1933.	Alivo 7th August 1932.	258	п	5	26th June 1933.	Alive 7th August 1933.	
453	II	5	26th June 1933.	Alive 7th August 1933.	436	11	5	26th June 1933.	Alive 7th August 1933.	
237	III	5	26th June 1933.	Alive 7th August 1933.	277	III	4	14th October 1932.		
174	īV	5	26th June 1933.	Alive 7th August 1933.	391	10	5	26th June 1933.	Alive 7th August 1933.	
381	V	5	26th June 1933.	R. *13 days.	331	V	5	26th June 1933.	1	
420	v	4	14th October 1932.	R. *16 days.	307	·v	1	17th November 1931.		
362	. VI	4	26th June 1933.	Alive 7th August 1933.		VI	1	17th December 1931.	R.* 16 days.	
292	VII	4	26th June 1933.	Alive 7th August 1933		VII	3	2nd Novem ber 1932.	D.(?')† 24th No	
36	ı VII	3	14th Octobe 1932.	r R.* 17 days	375	VII	y yar	2nd November 1932.	1932.	
19	2 VII		26th Jun 1933.	e Alive 7t August 1933		ı vii		2 20th Jul 1932.		

<sup>\*</sup> Rabies.

<sup>† (1) =</sup> Rabies not proved.

TABLE XI.

### Summary.

	Vacci	nated	Con	trols
<del></del>	Number	Per cent.	Number	Per cent.
Dogs	20		20	
Died of other causes (definite)	0	•••	1	*** *
Balance	20	•••	19	• # 1
		× .		-
Died—rabies proved	10	- 50	* 11	58
Rabies symptoms; Negri bodies not found; inoculated rabbits survived more than 100 days; rabies not proved.	1	•••	4	••• =
	_	-		
Total died .	11	55	15	79
		-		
Alive 7th August 1933	9	45	4	21

Our latest experiments have indicated a possibility of the chloroform-treated vaccine being somewhat effective as an immunizing agent against certain strains of street virus. There possibly has been some indication that exposure to some strains of street virus builds some resistance to later exposures. Generally speaking, however, dogs which have resisted exposure to a comparatively large dose of a potent virus have later succumbed to rabies infection when exposed to a comparatively small dose of virus. Some dogs succumbed after resisting several exposures.

Dog.	Exposed.	Virus.	Method.		Dose (c.c.).	Died.	Days
18	20th November 1928	27993	Intramuscular		1·0 0·25	21st December 1928 20th December 1928	31 30
. 25 33	20th November 1928 20th November 1928	27993 27993	Intravencus Intraperitoneal	:	0.25	6th January 1929 .	47
21 95	20th November 1928 20th November 1928	27993 27993	Intravenous Intravenous		1.0 0.5	30th December 1928 29th December 1928	39
108 81	20th November 1928 18th April 1929	27993 29995	Intramuscular Dog bite	•	0.25	30th December 1928	40 73
239	19th April 1929	29952 27039*	Subarachnoid	4	0·25 1·0	13th June 1929 . 8th July 1919 .	58
240 307	19th April 1929 17th November 1931	B71	Intramuscular	:	1.0	8th January 1932 .	52
357 232	20th July 1932 22nd September 1932	B88 58982	Subarachnoid Subarachnoid	:	0.2	24th August 1932 . 3rd May 1933 .	223
262	2nd November 1932	58982	Subarachnoid		- 0.2	5th December 1932	33

<sup>\*</sup> Submaxillary gland extract.

Table XIII.

Data on rabbits with prolonged incubation periods.

Rabbit.	Exposed.	Virus,	Method.	Dose (c.c.).	Died.	Days.
7190 7198 7288 7290 7594 8294 10499 10503 10996 12193	20th November 1928 22nd November 1928 28th December 1928 28th December 1928 15th March 1929 20th November 1929 17th November 1931 17th November 1932 15th April 1932 5th May 1933	27993 27993 27993 27993 29870 33795 50012 50012 1850EE 58982D232	Intraocular Subdural Subdural Subdural Subdural Subdural Subdural Subdural Subdural Subdural	0°25 0°25 0°25 0°25 0°25 0°25 0°20 0°20	14th February 1929 29th December 1928 22nd July 1929 3rd February 1929 31st May 1929 29th December 1929 2°nd January 1932 16th February 1932 13th June 1932 24th July 1933	86 37 206 37 77 39 66 91 59 80

A few dogs in both the vaccinated and control groups have resisted numerous exposures. It seems that if a dead virus will successfully immunize against rabies, a stronger resistance should exist in dogs which have survived successive exposures to a living virus.

Quite frequently cases of rabies have been reported in vaccinated dogs which were exposed to the bite of a rabid dog previous to vaccination, but very little data have been furnished on dogs which have been exposed to natural infection subsequent to vaccination.

In our experiments, all inoculated dogs were kept in individual locked cages made of wire cloth. No cases of rabies developed in healthy dogs which were placed in cages previously occupied by rabid dogs, and no cases developed in healthy dogs kept in separate cages in close contact with cages containing dogs showing physical symptoms of rabies.

There was no evidence in any case of harmful effects following the use of vaccine. All vaccinated dogs remained healthy from the standpoint of rabies until after exposure to rabies virus.

#### CONCLUSIONS.

Conclusions at this time can be no more than tentative.

Our experiments indicated that the carbolized vaccines used did not immunize against rabies.

The experimental use of chloroform-treated vaccine has offered somewhat more encouraging results than the carbolized vaccine, but not sufficient to warrant confidence in it to the exclusion of sanitary and police measures. Apparently no harmful effects are caused by the vaccine, and it is doubtful if enough good can come from its use to justify the expense involved.

Sanitary officials should not rely on vaccination as a means of rabies control.

Effective quarantine has successfully controlled rabies in districts where enforced.

All dogs known to have been exposed to rabies should be killed.

The usual 100-day quarantine does not furnish positive assurance that cases of rabies will not develop later, although for practical purposes it probably should not be increased.

There undoubtedly is a successful way of immunizing dogs against rabies, but neither the proper method nor the proper vaccine seems to have been found.

Several directions of future study have been indicated by past results, and we hope to continue our work on canine diseases, but we are not encouraged with the results obtained in testing the immunizing properties of canine rabies vaccines now on the market.

#### REFERENCE.

Barne, M.F., Metcalfe, A.N., and Lentz, W. J.: Investigations of canin reference to rabies. Preliminary report. *Jour. A.V.M.A.*, 1r pp. 34-52.

### ABSTRACTS

The present status of anthelmintic medication for gastro-intestinal parasites of the horse. Wright, W. H. J. Amer. Vet. Med. Assoc. LXXXIV, 11-24 (1934).

In spite of a considerable demand there is scarcely any drug or combination of drugs which is effective both for removal of bots and ascarids on the one hand and the Strongyles and Cylicostomes on the other.

Method of Administration. The stomach-tube is the method of choice. For parasites of stomach and small intestine fast for 18-24 hours, for those of large intestine fasting for 36 hours is necessary. The usual procedure is to administer treatment twice a year.

Contraindications for treatment. General. Very young or very old, weak animals and those suffering from febrile symptoms. The treatment of animals suffering from severe parasitic infestation should be discretionary.

Contraindications in the case of the following drugs are stated against them.

Carbon disulphide. Animals suffering from gastro-enteritis, gastric or intestinal colic. Advanced pregnancy. Fats and oils should be avoided.

Carbon tetrachloride. Icterus, hepatitis, cirrhosis of the liver. Animals with low blood calcium content.

N-butylidene chloride. Chronic constipation.

Oil of chenopodium. Constipation, gastro-enteritis, febrile conditions, pregnancy, highly bred animals of nervous temperament as well as phlegmatic animals of ordinary breeds.

Tetrachlorethylene. Although this drug is less effective it may be used in case where carbon tetrachloride is contraindicated.

#### Specific therapy.

Tapeworms. Specific drugs are oil of turpentine, arecanut, kamala and oleoresin of male fern.

Boots, Stomach worms and Ascarids. Carbon disulphide is the best drug.

Strongyles and Cylicostomes. Oil of chenopodium is very effective.

Pin worms. Drugs effective are oil of chenopodium and oil of turpentine. [ G. D. B.]

### Recent advances in the knowledge of anthelmintics. Mönnig, H. O. J. S.-Afri. Vet. Med. Assoc. 4,206-209 (1933).

exposur... way to study anthelmintics is to proceed in a systematic way by building up a a stronger resusuration and testing their properties. In this manner the drugs such as carbon to a living virus rethylene, N-butyl and N-butylidene chloride were discovered. Another way problem is by studying the metabolism of the parasites. For example tapeworms

Quite frequent of calcium. A suitable calcium compound having an anthelmintic action may were exposed to hich will be readily absorbed by them and thus kill them.

data have been furnished bed. This drug is safe for sheep, goats and poultry, but is highly toxic subsequent to vaccination

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Tetrachlorethylene. This drug is much safer than earbon-tetrachloride, especially in cases of young animals.

Hexachlorethylene. This seems to be the best drug known at present for liver flukes in cattle. It however taints the milk slightly.

N-butylidene chloride. Removes ascarids and hookworms of the dog, strongyles of horses and ascarids of fowls.

Hexyl-resorcinal. This is a useful remedy for ascarids, hookworms and pinworms of man and nodular worms in sheep.

Hexyl-mets-cresol. This drug has all the good and none of the bad qualities of hexylresoreinol.

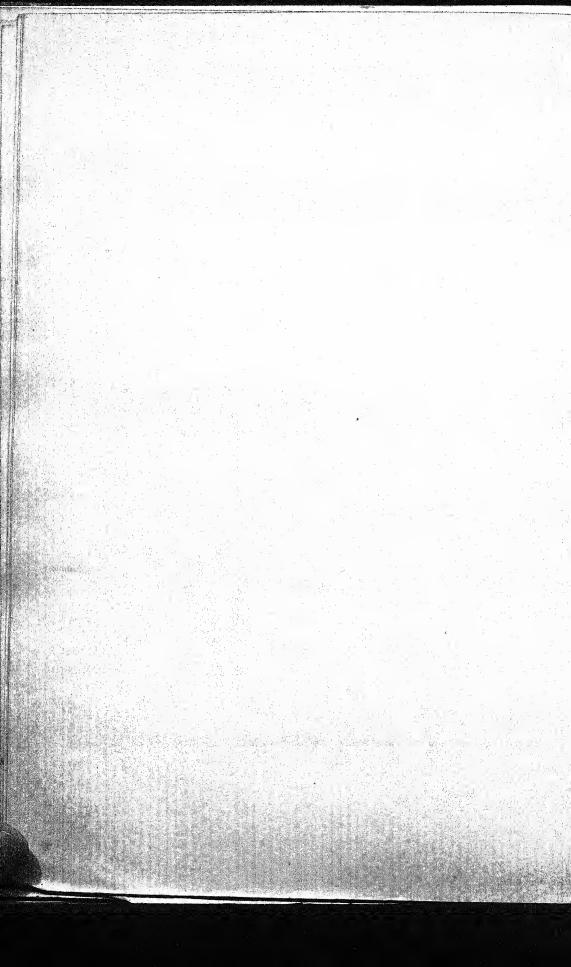
[ G. D. B.]

The effect of nematode infestation on mineral metabolism. Shearer, G. D. & Steward, J. 3rd Rep. of Dir. Inst. Anim. Path. Univ. Cambridge, 87-129 (1933).

It has been experimentally shown that heavy nematche infection in lambs in the 4th stomach or the intestine retards protein digestion in animals, but has no effect on the other nutritive components such as other extracts and nitrogen extractives.

The nematode infestation interferes with the calcium and phosphorus metabolism of the animals, but has no effect on the sedium and potassium metabolism.

A substance called "Nezyme" extracted from the nematodes of sheep is shown by experiments in vitro to inhibit the action of pepsin. The excretion of this substance renders the animals incapable of fully digesting the proteins which eventually brings about their emaciation and poor condition in general. [G. D. B.]



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<sup>\*</sup> Agriculture and Livestock in India.' 'The Indian Journal of Agricultural Science' and 'The Indian Journal of Veterinary Science and Animal Husbandry' replaced the following publications of the Imperial Department of Agriculture in India with effect from January 1931—

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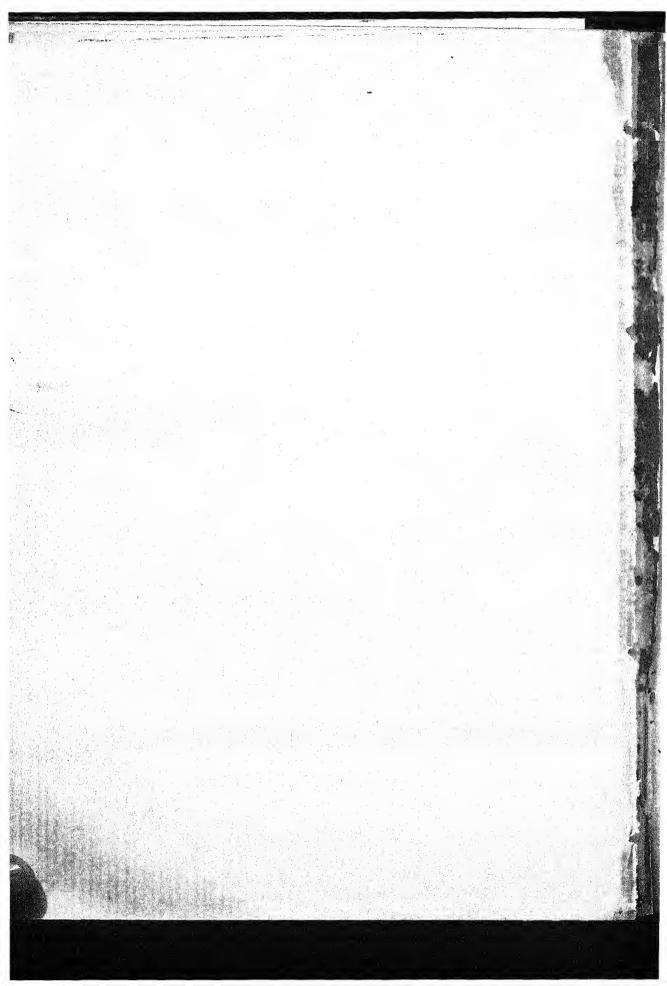
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(Vol. IV, PART IV)

December, 1934.

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### ORIGINAL ARTICLES

## STUDIES ON THE DETERMINATION OF DIGESTIBILITY CO-EFFICIENTS.

I. A NEW METHOD OF EXPERIMENTATION AND COMPUTATION FOR DIRECTLY OBTAINING THE DIGESTIBILITY CO-EFFICIENTS OF INDIVIDUAL FEED NUTRIENTS IN A MIXED RATION.

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(Received for publication on 28th August 1934.)
(With eleven charts.)

### INTRODUCTION.

The first step in any digestion experiment is to determine as accurately as possible what proportion of the different ingredients constituting the ration is actually capable of digestion. The usual method consists in feeding the animal a known weight of a certain feed, then estimating the amount voided in the faeces, the difference being assumed as equivalent to the amount digested. This procedure dates back since its inception by Henneberg and Stohmann (about 1860) and still forms the basis of all digestion trials; and while it has served fairly well for all practical purposes, the estimation of individual digestibilities in a mixture of two or more feeds has naturally been a matter beset with complexities, since there is no method still known by which the digestibility of each feed could be directly and separately evaluated.

In the case of a single feed the estimation of the digestibility on this basis should not theoretically offer any great difficulty. At any rate eminent investi-

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gators like Kellner and Armsby have described the estimation of clover hay and meadow hay respectively on this basis and their statements do not refer to any serious difficulty, although with a combined feed the latter has remarked that "the determination of digestibility of a concentrate in this way is less accurate and the range of uncertainty thus introduced may be very wide". The present authors have found that apart from such difficulties (in combined feeds) the results obtained with a single feed like paddy straw also have varied widely; and in the case of the protein fraction the matter has been further complicated with a large number of negative values. With the addition of a concentrate and working with assumed values of published standards for the latter, the results have also not been satisfactory; and as will be explained later on, it seems doubtful if outside standards are suitable under the conditions of our experiments.

These difficulties led to the trial by another method of experimentation based on the graphical analysis of the data; and the results so far appear to hold out a promise of a greater degree of accuracy. As a natural corollary to the graphical solution, multiple regression equations were later on used at the suggestion of Professor P. C. Mahalanobis and Mr. S. Bose of the Presidency College, Calcutta, and the present paper deals with the various aspects of these results.

It is necessary at this stage to touch briefly on some of the salient points leading to the initiation of the methods.

With the start of the Animal Nutrition Section at Dacca by a grant from the Imperial Council of Agricultural Research, India, the first attention was necessarily directed towards the behaviour of paddy straw since it formed the staple fodder of the province.

Experiments were conducted on bullocks which were more or less representative of the type of animals used throughout the province. The reason for this was that any results obtained which could be reasonably accepted as reliable could then be applied to the cattle population of the whole province.

As pedigreed animals are rare in Bengal (even the Departmental herd might not yet be accepted as such) and also as such animals are somewhat inclined to delicacy in feeding it was considered that it was better to use the ordinary country animals than to have recourse to pedigreed herd of the Department.

Experiments with paddy straw as the sole feed as well as in combination with other feeds were started. The object was amongst others to determine the digestibilities of the different feeds, both singly and in combination by the method in vogue, viz., the estimation of the digestibility of the single feed, say, paddy straw at first, then repeating the experiment with the addition of a concentrate, say, linseed cake, the computation of the second being made by a corresponding deduc-

tion of the values of the first, or, as is often done by applying the published values of "known" standards for the concentrate and then calculating by elimination the digestibilities of the roughage.

### ROUGHAGE AS A SINGLE FEED.

When a roughage like paddy straw is used as a single feed, the insufficient supply of the protein fraction in it in particular appears to be seriously reflected on the animal system, in consequence of which not only is there an adverse mtrogen balance but sometimes even the faeces show more nitrogen than is supplied in the feed. The result is that the digestibility of protein often records a negative value. We conducted six digestion trials and their main features are set forth in the following table, and for comparison some figures of Warth [1923] and Lander and Dharmani [1931] are also incorporated.

TABLE I.

Co-efficient of digestibility of paddy straw when given as a single feed.

THE RESERVOIR	Participation of the participa	NOTICE OF STREET	MATERIAL PROPERTY.	CONTRACTOR DESCRIPTION OF THE PROPERTY OF THE				
Garganinssonduma	-	Date	Exp.	Animal	Dry matter	Crude protein	Ether extract	Crude fibre
1	2	3	4	5	6	7	8	. 9
· ·		19-5-1932	IV	$D_7$	47.93	+5.86	55.32	64.57
	Dacca	18-7-1932	XII	D,	35.69	-31.26	48.00	56.21
Bengal .		12-6-1932	VIII	D <sub>8</sub>	43.69	-3.74	49.26	59.29
		18-7-1932	XII	D <sub>8</sub>	28.92	35.87	34.49	45.57
	Rangpur {	10-8-1932	XIII	R <sub>1</sub>	52.24	<b>—7</b> ·22	36.00	69.95
	. (	10-8-1932	XIII	$R_2$	46.21	<b>—7·71</b>	25.58	62.78
Pu	sa (	1923	VI	Kailash	51.30	-2.82*	59.90	75.50
Table IV & V	of original)	1923	22	Mahadev	46.90	-3.05*	59.90	72.10
Lyall	Inur	9-11-1929	••	Heifer 122	41.79	-14.29	40.00	60.30
Kangra rice straw) Table VI of original.		9-11-1929		Heifer 128	40.83	-25.00	50.00	57.84
		9-11-1929	]	Heifer 133	45.56	18·18	50.00	64.03

<sup>\*</sup> These two figures are actual amount (in gms.) representing digestion and not the digestibility co-efficients. The negative aspect is the main point shown here.



In this experiment (with paddy straw as the sole feed) the feeding was carried out for four months and eighteen days (11th April to 28th August 1932) at Dacca and for four months (25th April to 25th August 1932) at Rangpur. At Dacca the digestion and metabolism experiments were conducted twice while at Rangpur only once. The two tests on the same animals at Dacca has enabled a better comparison of the fluctuation of digestibility and it will be noted that in the case of D<sub>7</sub>, during an interval of two months between one test and another, the digestibility (of dry matter—to take one component) showed a decline from 47.93 per cent. to 35.69 per cent. (Column 6, Table I) or 25.54 per cent. over the first digestion, whereas in the case of D<sub>8</sub> during a course of five weeks' interval it has gone down from 43.69 per cent. to 28.92 per cent. or 33.81 per cent. over the first digestion. The protein figures have been negative in all except in one test and the digestion of all other components have gone down naturally reflecting in a gradually decreasing availability of total digestible nutrients and net energy (Table II, Columns 4 and 6).

-]	_
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ABLE	
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	1							
	Loss	gan (whole period) 10	lbs. 85·1	•	79-3		37.5	41.1
Live weight	At the ond	28th August **** 9	lbs.	:	310-7	25th August	333.1	334.3
	At start	11th April *** S	lbs. 402·8	•	390.0	25th April	370.6	375.4
Not energy per day in therms	Required at 6 therms	per 1,000 lbs. Live weight 7	\$÷00.6	%*F6.7	3.01**	2-88**	5.03**	2.92**
Net energy ther	Actual	obtained 6	1.64	0.87	1.24	0.40	1.97	1-74
Nutritive	ratio (round figure).	າລຸ	1:238	-1:39*	-1:362*	—1:27 <b>*</b>	-1:230*	-1:193*
Total	digestible nutrient per day	<del>~11</del> .	lbs. 2.76	1.91	2-25	1.36	76.7 7.04	2.69
	Crude protein digested	ന	gms. +5·228	-22.757	9.788	23-365	-5.832	-6.350
*	Period	<b>©1</b>	1st Test	2nd Test	1st Test	2nd Test	•	:
pro-	Animal No.	П	, A		n n		$\mathbb{R}_1$	R2 .

\* The negative calculation has been preferred to show the numerical extent of negative proportion with respect to protein.

\*\* These values have been calculated on the weights recorded during the periods of test and based on the proportion of 2/3rd power of

live weights.

† This table read with graph (I) will show that there is a marked tendency of the lowering of the digestion.

\*\*\* First week's average.

\*\*\*\* Last week's average.

In Table II the actual net energy obtained during the periods of tests and the required net energy (column 7) have both been shown. In calculating the latter, Warths' figure [1926] of 6 therms per 1,000 lbs. live weight has been assumed. On this basis D<sub>7</sub> should have received about 2.9 to 3.1 therms per day (Column 7), but instead it had only 1.64 therms during the first test, which came down to 0.87 therms during the 2nd test. In the case of D<sub>8</sub> its net energy dwindled down to 0.4 therms (during the 2nd test) or less than one-seventh of the actual requirement. All these had their reflex in the total fall of live-weights (Column 10, Table II, also Graph I). In the case of R<sub>I</sub> and R<sub>2</sub> the difference is only in degree, otherwise the tendency of progressive decline has been working in all. The nitrogen balance too has been equally characteristic of the grave nature of the deficiency in supply; and it will be noted that the longer the experiments continued, the greater was the rate of daily loss of live-weight (Table III, Columns 7 and 8). The fall in live-weights is also shown in chart I.

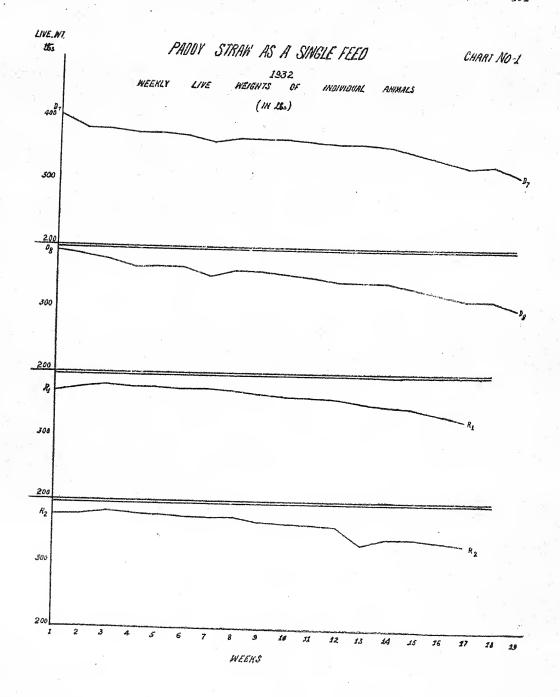


Table III.

Nitrogen balance per day.

Date	Animal No.	In paddy straw ingested	Voided in faeces	Digested	Voided in, urine	Balance	Loss or gain in Live weights per day
7	2	3	4	5	6	7	8
19th to 28th May 1932.	$D_{7}$	gm. 14·271	gm. 13:434	gm. +0°837	gm. 7·040	gm. —6·203	lbs. —0·0
18th to 27th July 1932.	D,	11:649	15-533	-3.884	5.706	<b>9</b> ∙590	-2:7
12th to 21st June 1932.	$\mathbf{D}_{\mathbf{z}}$	11.912	12:358	0·443	5.861	-6:304	-1.0
18th to 27th July 1932.	$\mathbf{D}_{\mathtt{g}}$	10.423	14.162	-3:739	4.536	8·275	-2.3
10th to 19th August 1932.	$\mathbf{R_1}$	12:924	13.857	-0.933	5.444	-6:377	1.0
10th to 19th August 1932.	$\mathbf{R_2}$	13·181	14.197	-1:016	5.150	-6:166	-0.7

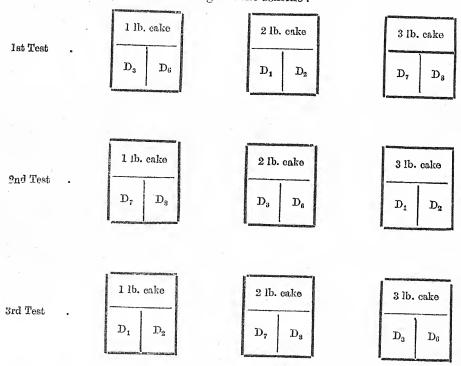
The Need of Balanced Ration. In such circumstance the addition of a concentrate becomes a necessity in order that the experiment can be conducted under more normal conditions where the animals could have at least a balanced ration. But with a mixed ration (here one roughage and one concentrate) the necessity arises to assume that the digestibility of one of the feeds is known and constant, so that by corresponding elemination we may calculate the digestibility of the other. Since however such digestibility figures for India are not available, we had to rely on "known" published data of elsewhere (European or American) and thereby, making the corresponding deduction, assign the digestibilities of the roughage.

### EXPERIMENTAL SCHEME,

Our investigation naturally included this part of the trial (viz., calculation by assumed values) and in order to combine in the same the other objectives now

leading to this paper, a fairly elaborate scheme was set up, in which it was ensured that the normal condition of the animals was maintained. This will be seen from the nitrogen balance and live weight figures, etc. (Appendices I and II). Six animals were divided into three groups, each group receiving in turn 1 lb., 2 lbs. and 3 lbs. linseed cake as concentrate with paddy straw ad lib as roughage. The animals were so chosen that at the time of starting the experiment the total live weight under each group was more or less the same. Further sufficient interval was provided between one test and another in order to eliminate as far as possible the residual effect of the previous feed. Sufficient quantities of straw and cake from the same source were stored in advance to ensure a uniform quality of feed during the entire period of the trial.

The following represents the design of the scheme:—



The whole arrangement was on a restricted randomised basis distributed in a cyclic order whereby all the animals were in turn under 1 lb., 2 lb. and 3 lb. combinations. This provided on one side, the same kind and quality of both roughage and concentrate and on the other, reduced the chances of error to a minimum whether with respect to the animals or their feeds.

As will be evident from the above, the design of the experiment provided (in three cycles) 18 individual tests, six with 1 lb., six with 2 lb. and six with 3 lb. cake. It was felt however that the extension of the experiment for another complete cycle of 18 tests would have produced a more satisfactory material for computation but pressure of work and limitation of staff did not permit it.

The objects of the scheme were mainly three-fold:-

- 1. The estimation of digestibility by the "method of elimination", i.e., by applying the assumed values of one of the feeds, to work out the digestibility of the other.
- 2. The direct estimation of digestibility by the graphical method.
- 3. The direct estimation of digestibility on the basis of multiple regression equations.

### METHOD OF ELIMINATION.

For this purpose the assumed values were used from Henry and Morrisons' "Feeds and Feeding" [1928].

As already stated this procedure implies the carrying out of the experiment by the addition of a concentrate whose digestibilities are supposed to be "known". With the 18 individual trials, six with 1 lb., six with 2 lb. and six with 3 lb. cake, and working out by difference the digestibilities of the paddy straw, we obtained 18 figures for each component which varied in the case of dry matter from 38 to 49 per cent.; for ether extract from 35 to 69 per cent.; for crude protein from a positive figure of 11 per cent. to a negative value of 13 per cent. In order therefore to reduce the variations to a minimum, each set of 1 lb., 2 lb. and 3 lb. combinations was worked out into its respective mean. The figures are set forth in the following table.

TABLE IV.

Digestibilities of paddy straw worked out by the aid of Henry and Morrison's standards for cake.

	Dry matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract
Mean of 1 lb. group	44.28	+5.91	48.60	60.69	45.29
Mean of 2 lb. group	42.05	-1.27	53-24	58.33	42.15
Mean of 3 lb. group	41.83	-0.91	59•37	58-25	42.69

The most noteworthy points about these figures are the values of the protein on one side and the ether extract on the other. When the amount of concentrate was least, viz., 1 lb., the protein digestibility of paddy straw worked out at a higher and positive figure, viz., 5.91 per cent., whereas with increased amounts of cake the figures were not only lower but definitely negative. This is suggestive of the fact that a higher value was assigned to the share of cake, leaving a low or negative value in favour of paddy straw. In the case of the ether extract the position was re-With increasing doses of cake the digestibility has definitely increased, viz., 48.6 per cent., 53.24 per cent. and 59.37 per cent. in the 1 lb., 2 lb. and 3 lb. groups respectively. Or in other words a lower value in the case of ether extract was assigned for linseed cake, thereby leaving a higher margin in favour of paddy That this was probably the most likely cause is borne out by the figures of cake digestibilities obtained by the graphical method as well as by multiple regression equations. In the following table the results obtained by all the methods are given.

Table V.

Digestibility of linsecd cake.

			Minerity.					Henry and Morrison	Graphical method	Multiple regression equation
Dry matter	•	•	•			•	.	79	70.10	65.15
Crude protein	•	•	a		•			89	84.75	84.11
Ether extract		٠	4.1					89	96-36	- 20
Crude fibre								57		95-60
Nitrogen free ext	rant					•	- 1		27-29	7.71*
B	LCUCI	•	•	•	•	•		78	67-30	61.34

It will be noted that whether according to the worked-out figures of paddy straw (Table IV) or on a comparison with the figures by the new methods (Table V) the assumed cake digestibility figures of Henry and Morrison appear definitely higher for all the components except that of ether extract in which case it is considerably lower. These have been correspondingly reflected in a progressive rise and fall in almost all the figures of paddy straw under the 3 sets of 1 lb., 2 lb. and 3 lb. combinations (Table IV), the greatest difference occurring in the cases of ether extract and crude protein, the latter giving also negative values. Such differences with protein in particular seem to have also been obtained by Warth and Gossip [ 1928 ] and Warth [ 1923 ] as are shown in the following table.

<sup>\*</sup> This is the only value which has not been found satisfactory. The cause of it has been discussed later.

TABLE VI.

Nitregen digested from paddy straw (Experiment at Karnal and Pusu)

(Original Tables XIV and V).

					(Wa	Karnal arth and Gos	sip)		usa arth)
					Ration 1	Ration 2	Ration 3	Kailash	Mahdeva
	1				2	3	4	5	6
Nitrogen in ration				:	45.88	59.13	72.33	-	general transmission of the second of the
Nitrogen in faeces	1.8				19:30	21.50	23.50		-
Nitrogen digested	=				26.58	37.63	48.38		
Nitrogen digested fro	in cor	central	е		25.77	38.08	5 <b>0:3</b> 9		
Nitrogen digested fro	om str	aw	•		+0.81	-0.45	-1.51	3.01*	-1.78*

It will thus be seen that when the computation is made on the basis of assumed digestibilities for concentrates, the amount falling to the share of the roughage is evidently affected to a definite extent according as the values assigned for concentrate are more or less than the actual. In the progressive doses of concentrate as tried by ourselves on a more comprehensive scale, this rise and fall has behaved with a remarkable consistency indicating definitely which of the assigned values of the components of concentrate were above or below the actual amount.

It is however necessary to emphasise here the doubtful utility of the present unavoidable application of outside standards. In fact the disadvantages of the use of such data have also been noticed by the continental workers as well.

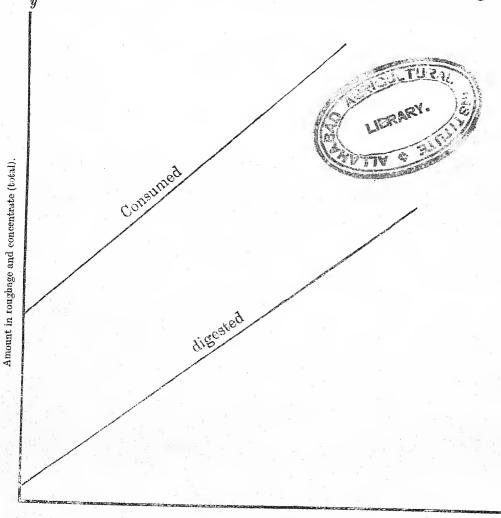
Wood [1914] of Cambridge University has shown that they were too high for an ox fed on a heavy fattening ration and Eckles [1] of Missouri Station has found such values too low when applied to the dairy cow. Our work also point to the unsuitability of their general application, but the paucity of local data left us little alternative; and in consequence the digestibilities of many of our feeds have of necessity to be worked out with these assumed figures obtained under conditions necessarily different from ours. But the variability in the values all the more emphasises the inexact nature of working by "the method of elimination". Any attempt therefore to evolve a more suitable method would solve a long-felt difficulty even if it be only a partial improvement over the prevailing one.

<sup>\*</sup> Warth's results contain a number of such negative values but only two are quoted here.

THE DIRECT ESTIMATION OF DIGESTIBILITIES OF BOTH STRAW AND CAKE BY THE GRAPHICAL METHOD.

As already stated, the unsatisfactory results obtained by the existing methods have led to its trial.

It is necessary to state here that this method was apparently given some trial by Warth [1929] on the aspect of protein digestion of concentrates. It has however been carried out in the present case on a rather more comprehensive scale including all the individual components comprising the roughage and concentrate. A brief description of the method as here used is given in the following.



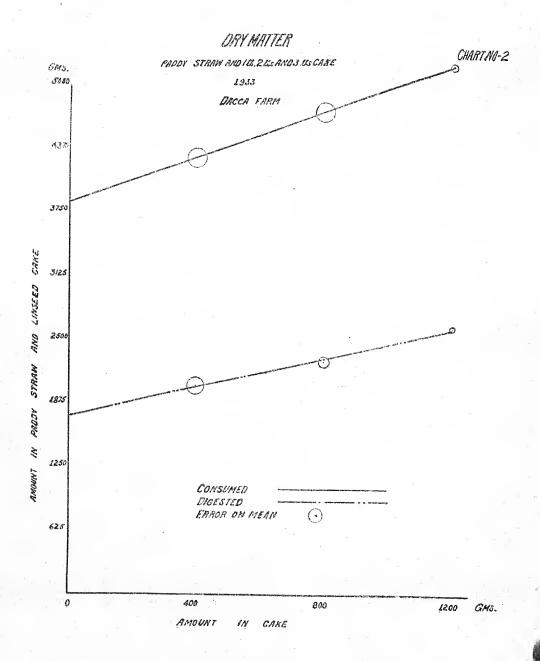
Amount in concentrate.

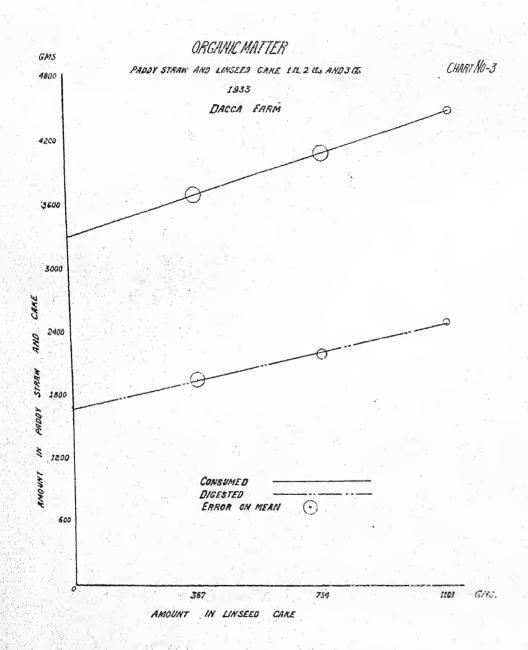
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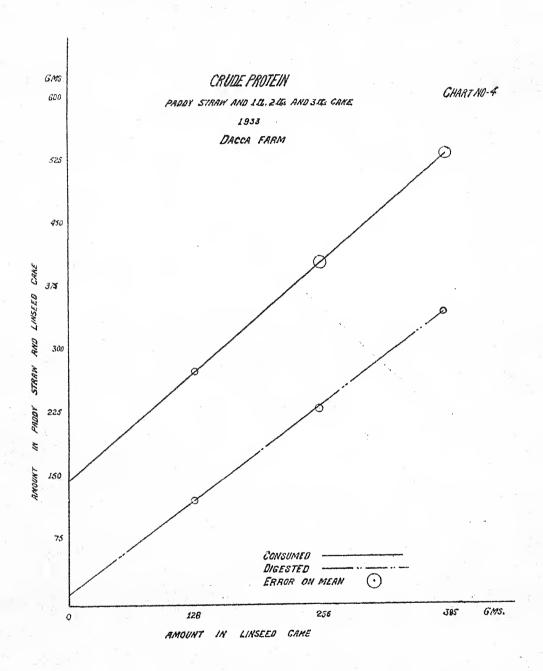
Let x axis represent the amounts of concentrate consumed and the ordinates the total amounts consumed and digested on the same scale. The upper line stands for total consumption and the lower line for total digestion (from both roughage and concentrate). The lines are produced to meet the y axis and the points at which they meet are carefully read. These have been shown in columns 2 and 3 of Table VII. Obviously they represent the amounts consumed and digested from the roughage (paddy straw) when no concentrate is supposed to be given. Their ratio is shown in column (4) representing the digestibilities of the components of roughage. Similarly the slope of the lower curve (column 5) stands for the digestibility of the concentrate.

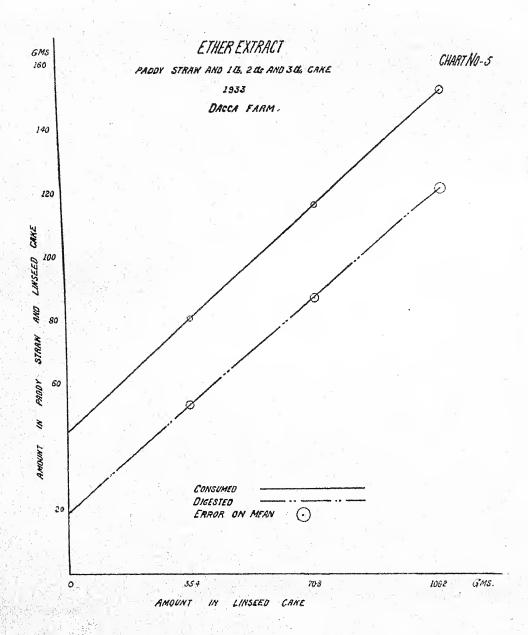
Each of the individual components, viz., dry matter, crude protein, ether extract, etc., has thus been separately plotted out under 1 lb., 2 lb., and 3 lb. combinations. These have been shown in separate graphs (Charts 2—7). In these the mean under each group with its circle of error is also represented to indicate the range of variation.\*

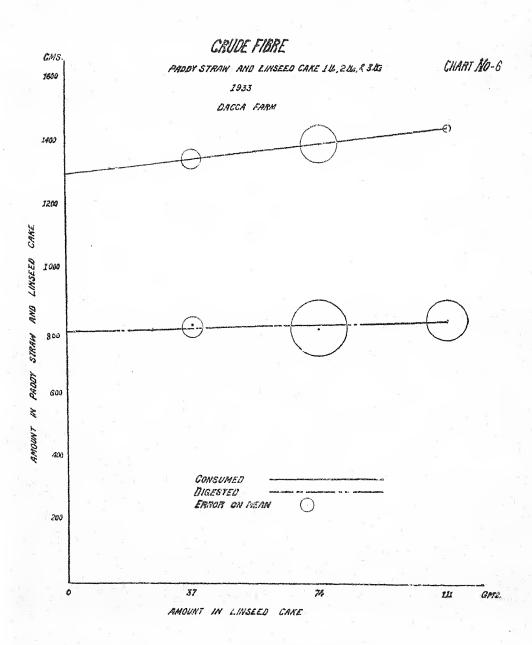
<sup>\*</sup> For accurate drawing the straight line of best fit can be obtained by least squares giving the ordinates as well as the slopes.

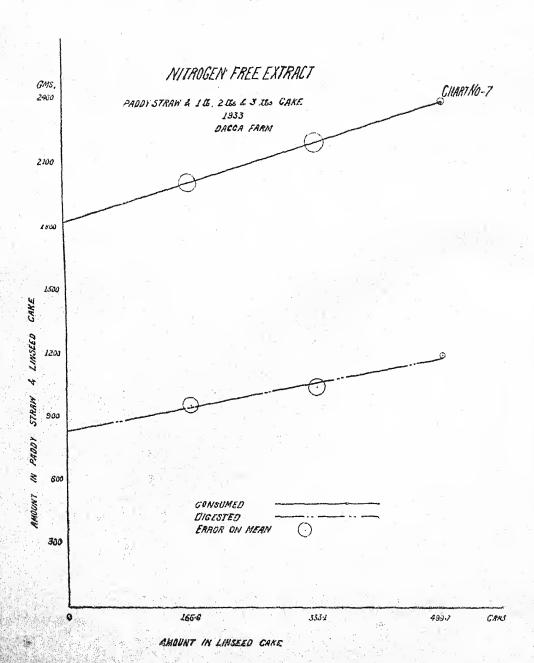












The values obtained from the graph drawn for each of the food components are set forth in the following Table:—

TABLE VII.\*

Comp	one	nts			Ordinate at $x=o$ (consumed) C g·ms.	Ordinate at $x=0$ (digested) D	Digestibility of roughage D	Slope of the curve (Digestibility of concentrate)
	1	······································			2	3		5
Dry matter	•				3,862	1,751	•4507	<b>'701</b>
Organic matter					3,326	1,687	•5073	·7216
Crude protein .	1				147	13	-0873	*8475
Ether extract	•		•		45	19	4316	*9636
Crude fibre .				•	1,302	803	•6165	•2729
Nitrogen free extr	act			•	1,830	845	•4617	•6730

<sup>\*</sup> The original data are reproduced in Appendix II.

It will be seen that the straight line in the graph has given a very close fit to the observed values except in the case of crude fibre †. This evidently points out that the interaction during the course of digestion is probably negligible and the digestibility is more or less constant for the three sets of combinations. This would suggest a fair order of reliability for the estimates of digestibility co-efficients.

THE DIRECT ESTIMATION OF DIGESTIBILITIES (OF STRAW AND CAKE) BY THE AID OF MULTIPLE REGRESSION EQUATIONS.

We have no direct way of ascertaining whether there is any or no interaction taking place during the course of digestion of cake and straw. The same state-

<sup>†</sup> In the case of fibre the range of variation of the observed values and the deviations from the straight line will be seen to be of the same order and hence the fit cannot be considered satisfactory.

ment holds good for the progressive doses of cake as used in our experiment. The graphical method however suggests that within the range of 1 lb., 2 lb. and 3 lb. combinations, the interaction at any rate would seem inappreciable. It may therefore be neglected for the purpose of this experiment, and as a first approximation be assumed that the behaviour of cake and straw with respect to their individual digestibilities is independent of each other and also constant. The subsequent analysis of the results, as will be shown later, supports this assumption within the limits of sampling errors in the case of the majority of the individual components of both the feeds.

A brief description is given in the following.

In the case of the above experiment with 1 lb., 2 lb. and 3 lb. cake as concentrate and paddy straw *ad lib* as roughage there is a mixed ration of two unknowns—viz., cake and paddy straw.

If now a and b represent the digestible fractions of the component (here crude protein) from straw and cake respectively, the linear relation is obtained as follows\*:—

y=Amount digested from straw and cake.

 $x_1$ =Amount consumed from straw.

 $x_2$ =Amount consumed from cake.

If the equation gives a good fit, it should reasonably follow that the hypothesis holds good and that the values of a and b thus obtained represent the nearest approach to the fractions digested from the respective share of  $X_1$  and  $X_2$ .

From the equations thus obtained, the expected values (y) of the amount digested from the observed values of  $x_1$  and  $x_2$  can be calculated. As an illustration the observed and expected values of one of the important constituents, viz., protein nitrogen has been shown in Table VIII. For this the co-efficients of digestibility as worked out are:

Digestible crude protein in paddy straw.—9.90 per cent.

Digestible crude protein in linseed cake.—84.13 per cent.

The method of calculation is described in detail in Appendix IV.

<sup>\*</sup> Since when  $x_1 = 0$  and  $x_2 = 0$  the amount digested is necessarily Zero, therefore no constant has been added. From the observed values also the value of c is found to be negligible in all cases.

Table VIII.

Crude protein (Total nitrogen).

Name of	Amount di	gested	Difference between		
bullocks.	Calculated $x_1 a \ x_2 b$	Observed $y$	calculated and observed		
1	2	3	4		
$\int D_1$	18.88	19.14	0.26		
$D_2$	18-91	19.61	-0.70		
$\bigcup_{1 \text{ lb. }} \mathcal{D}_{\mathfrak{d}}$	20.13	21.53	-1.40		
D <sub>6</sub>	20-14	21.21	-1.07		
$\mathbf{D}_{7}$	19.87	20.08	0.21		
$D_{\mathbf{a}}$	19.79	16:98	+2.81		
$\int D_1$	37.90	38.09	-0.19		
$\mathbf{D_2}$	37.75	37-50	+0.25		
$D_3$	37.62	36.70	+0.92		
D.	37.74	<b>37·</b> 93	-0.19		
$D_7$	35.07	34.38	+0.60		
$\mathbb{D}_{s}$	35-20	32.95	+2.25		
$D_1$	55.48	55.75	0.27		
$D_2$	55:41	53.09	+2.32		
$\mathbb{D}_{s}$	51.77	52•44	0.67		
$D_6$	51.73	5 <b>3·7</b> 8	-2.05		
$D_7$	55.27	56.90	-1.63		
$D_8$	55.35	55:35	0.0		
Total	. 663:41	663-41			

It will be seen from a reference to columns 2 and 3 (Table VIII), that the assumption of the constant digestibilities of roughage and concentrate is clearly borne out from the remarkable agreement of the observed and expected values of the amounts digested.

Applying the same procedure, all the other components, viz., dry matter, organic matter, crude protein, ether extract, crude fibre and nitrogen free extract have each been separately worked out. The value of a and b for each of them are set forth in Table IX and the necessary statistical tests of significance have been shown in Appendix V.

Table IX.\*

Best values of a and b.

				×-	Digestibil paddy (a	straw	Digestibilities of linseed cake (b)	
					By regression equation	Graphical method	By regression equation	Graphical method
1			-		2	3	4	, 5
Dry matter .					0.4556	0-4505	0.6515	0.7010
Organic matter		•			0.510324	0.5073	0.670692	0.7216
Crude protein .			a		0.098985	0.0873	0.841132	0.8475
Ether extract .					0.4379	0.4316	0.9730	0.9636
Orude fibre ** .					0.618935	0.6165	0.077092	0.2729
Nitrogen free extra	et			•	0.463524	0.4617	0.613449	0.6730

<sup>\*</sup> See also Appendix V.

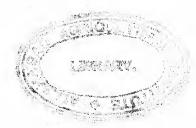
#### DISCUSSION.

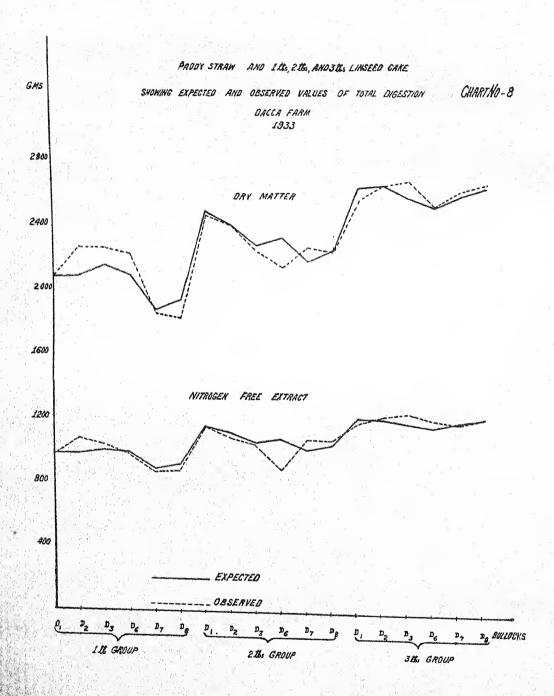
It has been stated earlier that as a first requisite for reliable digestibility figures the animals must be provided with more or less normal conditions and the feed must be at least a balanced ration. In the tests initiated by the authors these provisions were followed as far as possible. The critical examination that resulted with respect to the three methods, shows that in computing the figures by what has been termed "the method of elimination" the resulting values, worked out on the basis of assumed standards, are definitely affected according as the assumed values are higher or lower than the actual. The need for local data to replace these outside standards has become insistent. These difficulties chiefly account for the subsequent attempt, and the preliminary investigation appears to indicate that the working of data on the basis of linear relations enables a direct calculation and warrants a greater degree of

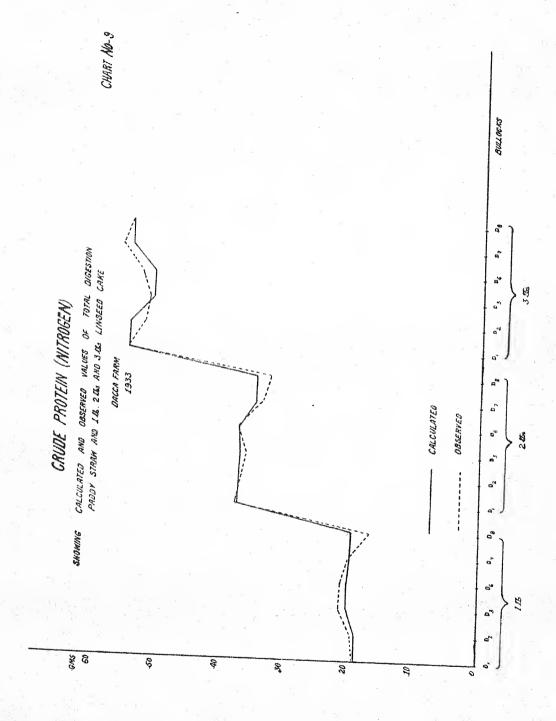
<sup>\*\*</sup> Vide remarks, page 305 foot note and also in discussion.

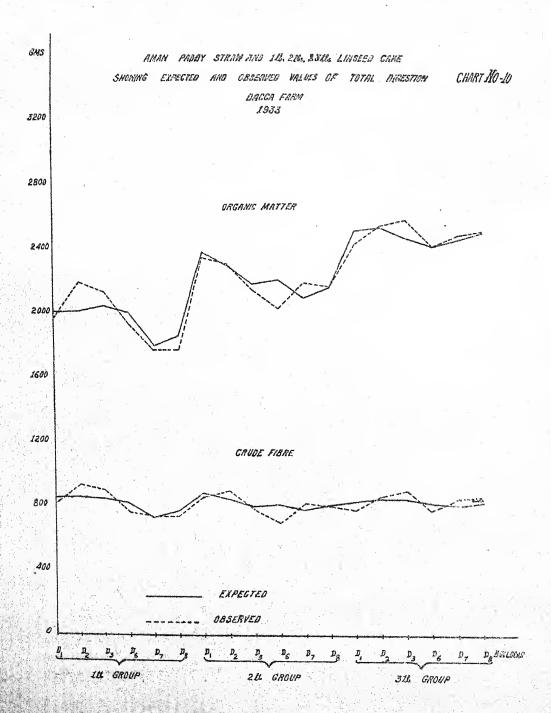
accuracy. The graphical method was thus brought in and as the next step, the multiple regression equations followed as a natural corollary to it.

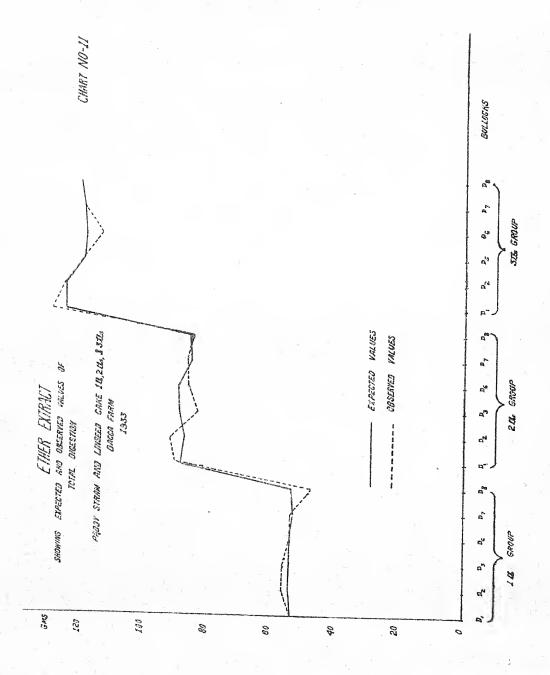
In order to get an idea of the nature of agreement obtained by this procedure the case of crude protein already given as an illustration of the working out of these methods might be taken up (Table 8, Columns 2 and 3). It will be seen that the actually observed values of digestion (y) and the calculated or expected values  $(x_1a+x_2b)$  worked out from the value of a and b (obtained by the application of regression equations) have recorded an unexpectedly remarkable coincidence of agreement. The co-relation (Appendix V, Column 8) has been above 99 per cent. and the observed ratio of variance has been much above the minimum requirement of Fisher's 1 per cent. point of distribution. The observed and expected values have also been plotted out (Chart 9) and it will be noted that the variation has been negligible. For the results worked out by the graphical method the reader is referred to Chart 4 (for protein) and Table IX. It will be seen that by the graphical method the errors on mean (also cf. Appendix III) have been very small and the agreement between it and that of regression equations has been reasonably close.











If the results of the other components, viz., dry matter, organic matter, ether extract, and nitrogen free extract (Appendix V) are examined it will be found that the co-relations are quite high with the observed ratios of variance quite above the minimum requirements of Fisher's 1 per cent. points of distribution. In these cases also the observed and expected (i.e., calculated) values of digestion has been separately plotted out (Charts 8, 10 and 11) to present a visible picture of the nature of variation. The results by graphical method have been shown in Table IX and Graphs 2, 3, 6 and 7 and also in Appendix III.

In the graphs the circles of error show the extent of deviation from the mean. These errors are on the actual values of consumption and digestion (Appendix III, Columns 4 and 6). Still they are quite small and would have appeared still less had they been shown in percentages. It should be stated here that although the graphical method cannot be as accurate as that by the application of regression equations, yet the agreement between both particularly in the case of roughage (paddy straw) has been remarkably close (Table 9, Columns 2 and 3 and also Appendix VII) with respect to all the components including even crude fibre.

With linseed cake also the agreement has been as good for protein and ether extract, but there has been some variation with dry matter, organic matter and nitrogen free extract. Yet taking into account the likely extent of variation inherent with the free hand drawing of the graphical method, the agreement can be said to be fairly satisfactory for all the individual components with the solitary exception of crude fibre in linseed cake which has been dealt with separately.

With regard to graphical analysis (Charts 2—7) it might be stated that despite its limitations it should form an indispensible adjunct to the regression equation method, as it serves to throw highly intersting side lights on many aspects. As an instance it may be noted that the mean consumption under all the three sets of combination has formed an almost perfect straight line in the case of all the components. The line showing the digestion has also passed either through or very close to the mean. Such an occurrence would strongly suggest that the relation is linear and the subsequent application of multiple regression equations is justifiable.

The small magnitude of errors obtained by the graphical method as well as the high co-relation values and observed ratios of variances obtained by regression equations, strongly suggest that the animals behaved normally and the extent of selective action within the limit of 1 to 3 lb. combinations of cake was not probably much or appreciable. This method will further enable us to ascertain whether there is any variation in the digestibility of roughage with the addition of different concentrates.

Crude fibre. This component demands special attention. Its digestibility co-efficient for paddy straw has been quite in agreement by both the graphical method and regression equation method. But in the case of linseed cake the graphical method has given 27.3 per cent. as the digestibility, whereas by the regression equation method the value has been 7.71 per cent.

In the graphical method however it is noted that the errors on mean (Chart 6) has been generally high particularly for the 2 lb. combination both for digestion and consumption. The straight line (Chart 6) representing digestion has passed almost horizontal with the x axis, indicating that with the increasing doses of cake the proportion of digestion increased very little. These factors have involved a high error reflecting in a wider variation by the two methods.

It may be stated that the results of crude fibre cannot be considered satisfactory. On the basis of regression equations it was the only component in which the co-relation was so low as 65.51 per cent. and the observed ratio of variance was below 1 per cent. points of distribution although within 5 per cent. distribution (Appendix V). As will be noted below (Table X bottom row), the mean fibre digestion (also indicated by Chart 6) has not been significantly different under different groups, i.e., from ration to ration. Hence its validity has been of a lower order.

Table X.

Crude fibre in total feed.

				Cr	ude fibre (Mea	n)
		1	•	1 lb. group gm.	2 lb. group gm.	3 lb. group gm.
Consumed	= (1)		Paddy straw Linseed cake	1311·237 36·967	1321·697 73·902	1330-002
Voided in fact Digested	.es		Total .	1348·204 530·365 817·839	1395-599 582-499 813-100	1440·871 602·865 838·006

The mean total amount ingested has been in increasing order with increasing doses of cake in the case of all the components including crude fibre (Appendix V) but while all the others have shown more or less an increasing amount of total

digestion, the digestion of this component has been practically stationary and possibly even lower. At any rate the figures under the 2 lb. combination would rather indicate a tendency of decreasing digestion; and if they are compared with the figures of 1 lb. combination it will be seen (Table XI)

TABLE XI.

	Crue	de fibre consur	ned	Voided in	Digested
	From straw gm.	From cake	Total gm.	gm.	gm.
1	2	3	4	õ	6
2 lb. combination (a)	1321.7	73.9	1395-6	582.5	813:1
1 lb. combination $(b)$	1311.2	37.0	1348-2	530.4	817.8
Difference $(a-b)$	10.5	36.9	47.4	52:1	4.7

that on the basis of difference between 2 lb. and 1 lb. combinations the increase in consumption of fibre was 47.4 gms., whereas the corresponding amount voided in the fæces was 52.1 gm., i.e., more than what was ingested. Thus there was a negative digestion of -4.7 gms. This difference is no doubt small, nor is it intended to be treated in the statistical sense; yet there appears to be some justification in referring to it, as this aspect taken with other factors has a bearing on a highly interesting side issue.

The behaviour of crude fibre seems to suggest that during the process of digestion some special phenomenon intervenes which reacts in such a way on the food material as to effect an apparent increase in the fibre fraction of faces or undigested residue, thereby showing an apparent decrease in the digestion. That such a probability is not unlikely seems to be borne out by the exceedingly interesting work of Woodman and Stewart of Cambridge [1932].

The fibre digestion of ruminants takes place by bacterial agency in the stomach, and these two authors have found by incubating linseed and other oil cakes with bacterial cultures that the ether soluble fraction of the oil cakes appears to produce some organic substance which is able "to resist solution by the reagents employed for the estimation of fibre". In consequence of this the amount of what is estimated as fibre is naturally increased in the undigested residue sometimes even more than what originally was present in the cake. In the

experiment by the above authors this increase has been such as to yield a negative value, viz., -1011 per cent. (in one case). These values have been termed by the above authors as "fermentation co-efficient" corresponding to the digestible coefficient of feeds. Our results also strongly suggest the probability of similar occurrence; and the rather divergent values of the digestibility co-efficients of crude fibre in linseed cake given by European and American authors might possibly be due to this hitherto unnoticed cause. Henry and Morrision [1928] have given the American value to be 57 per cent, and the same value of Kellner [1926] is 32 per cent. Not only is there a wide difference between these two values but according to Kellner the value oscillates between 0 per cent, to 92 per cent. Such a difference is suggestive of some phenomenon outside the normal course possibly of the nature indicated by Woodman and Stewarts' experiments. Armsby's statement [1930] also indicates that in a mixed ration of roughage and concentrate the value of crude fibre (and also ether extract) is much affected when the proportion is wide. But this statement following after a reference to meadow hay and maize meal does not seem to refer to oil cake and as such may not be quite applicable in the circumstances of this case; but the difficulties arising in his case are the main reason to cite it, for he states that "In extreme cases, absurd results are sometimes obtained such as negative digestibility or a digestibility greater than 100 per cent." In this experiment, the results shown in Table XI are also indicative of the tendency towards negative digestion and the very low value (7.71 per cent.) obtained by the regression equation (being based on the results of 18 tests) might possibly be explained by the probability that the individual digestions ranged between both negative and positive figures in a somewhat similar way as might possibly be the underlying cause for the values of 0 per cent. to 92 per cent. found by Kellner.

The chief causative factor for it is probably associated with the findings of Woodman and Stewart already noted. It might be stated here parenthetically that changes of a like nature were also apprehended by Armsby [1910], for he states "We have to take account of the possibility of conversion of members of one group of nutrients into those of another. For example, it seems not improbable that a portion of the crude fibre of feeding stuffs may be so modified in the digestive tract, without being actually dissolved, that in the fæces, it is determined as nitrogen free extract, thus diminishing the apparent digestibility of the latter group or increasing that of the crude fibre."

Such a phenomenon, whether like that suspected by Armsby or this, would stand in the way of accurate determination by any method not to speak of the one forming the basis of this paper. The matter is however under investigation. Leaving therefore this solitary exception of crude fibre, the results worked out by the new method have in all other cases conformed full. The temperature of statistical test.

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### CONCLUSION.

In digestion experiments the first condition, whether with one or more feeds, must be that the ration should on no account fall below a balanced supply. Given this condition, the next step is to calculate the digestibility from the amount consumed and the portion voided.

In a single feed the calculation is simple unless the feed is deficient like paddy straw, when complications arise on account of irregular digestion

In a mixture of more than one feed the computation based on what has been termed as "method of elimination" might affect the resulting values according as the assumed standards differ from the actual; and hence the use of such (outside) standards (based on conditions different from ours) might often be inapplicable as occurred in this case; but even when they are applicable they require to be verified, and above all local data under local condition are always preferable.

These considerations led the authors to try out other methods; and the experimental design and graphical method of analysis were the outcome of same. As a counterpart of the graphical method, multiple regression equations were subsequently tried and adopted. The chief advantage of both these methods are that they enable direct calculation to be done without having recourse to the doubtful alternative of using assumed values for a part of the feed.

To provide suitable data for calculation by these methods the experiment should be so designed as to ensure a reasonable number of tests in which all the animals selected should at one time or other, be under all the different doses intended for the investigation. The scheme undertaken by the authors with six animals was on a restricted randomised basis with I lb., 2 lb. and 3 lb. linseed cake; and was so designed as to enable eighteen individual tests in all, in which all the six animals in pairs or groups of two, were at one time or other under all the three combinations. This enabled uniform distribution of feed as well as all the animals, over the entire span of the experiment and also helped in the elimination of the largest possible avoidable errors. The design can be improved further, say, with more animals giving more groups.

The initial figures thus obtained from the experiments can then be used for the direct calculation of digestibilities either by the graphical method or by multiple regression equations, or both.

The results obtained by the authors have been quite encouraging, having conformed to the requirement of statistical tests in the case of all the components if we exclude the notable exception of crude fibre, since in this case extraneous causes appear to have intervened.

If this method stands up to subsequent trial, as it is confidently expected that it will, then the computation by its aid will remove a long felt difficulty.

The advantages of the method might be summed up as follows:—

- 1. Direct estimation of individual digestibilities in a mixture of more than one feed.
- 2. Dispensing with the present unavoidable alternative of using assumed values.
- 3. Study of same roughage under different concentrates and different roughages under same concentrates thereby enabling the determination of variability, if any, under these varying factors.

### SUMMARY.

- 1. A resume has been given showing the difficulties associated with the prevailing methods of calculating digestibilities in a single feed or in a mixture of more than one feed. The uncertain nature of the results when the feed is deficient in any one constituent has been emphasised.
- 2. The design of the experiment, as undertaken by the authors on a restricted randomised basis, has been shown with 1 lb., 2 lb. and 3 lb. combinations of linseed cake and paddy straw. The design can be further improved upon by a larger number of animals and tests.
- 3. The results with assumed digestibilities on the basis of outside standards have been worked out and discussed, showing their unsuitability of application in many cases as was also experienced by Woods and Eckles.
- 4. The need of verification of outside standards and preference of local standards for local feeds have both been emphasised.
- 5. The graphical method and process of working have been described and the graphs for each component have been shown in separate charts with the circles of error, and the digestibilities computed.
- 6. The multiple regression equations method following as a corollary to the graphical method has been described and the process of calculation shown (Appendix IV).

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- 7. Both graphical method and regression equation method enable direct calculation of digestibilities in a mixture of more than one feed and both have shown a reasonable order of agreement.
- 8. The digestibility of crude fibre with respect to linseed cake has been found to be the only instance which has shown discrepancy, but the cause of it appears to be associated with some bacterial action as indicated by the findings of Woodman and Stewart [1932].
  - 9. The chief advantages of both the methods are :-
    - (a) they enable direct calculation in a mixture of more than one feed.
    - (b) different roughage and concentrates can be separately worked out to study their behaviour on each other under various combinations and proportions.

## ACKNOWLEDGMENTS.

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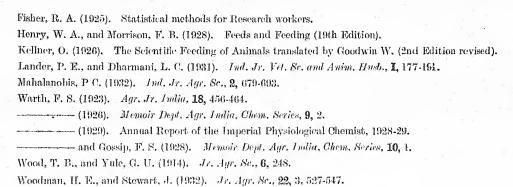
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# APPENDIX 1.

Aman paddy straw and 1 lb., 2 lb. and 3 lb. linseed cake. Nitrogen balance and live-weights (loss or gain).

	1 lb. com	bination.	2 lb. com	bination.	3 lb, con	bination.
Animal No.	Nitrogen balance grms.	Loss or gain in live- weight lbs.	Nitrogen balance grms.	Loss or gain in live- weight lbs.	Nitrogen balance grms.	Loss or gair in live- weight lbs.
1	2	3	4	5	6	7
D <sub>1</sub>	+0.966	-0.28	+10.309	-+1:20	+9.466	+1.300
D <sub>2</sub>	+1.572	-1.54	+8:325	+0.95	+6.255	+0.880
D <sub>3</sub>	- -4.804	+0.64	+2.980	+0.31	+9.147	+0.910
D <sub>6</sub>	+2.415	+0.41	+4.421	+0.48	4-9-240	+0.990
D,	+3.262	+0.43	+8.261	+0.71	+-15.740	+-1-230
D <sub>8</sub>	+0.369	+0.31	+4.519	+0.58	+14.441	+1.570

## APPENDIX II.

Digestible nutrients etc., received by each animal. (Paddy straw and 1 lb., 2 lb. and 3 lb. linseed cake).

1 lb. group.			2 lb. group	<b>p.</b>	3 lb. group.				
Animal No.	Total digesti- ble nutrients Ibs.	Nutritive ratio 1:	Net energy (Therms).	Total digesti- ble nutrients lbs.	Nutritive ratio 1:	energy	Total digesti- ble nutrients lbs.	Nutritive ratio I :	Net energy (Therms).
$\overline{\mathrm{D}_{\mathrm{I}}}$ , .	4.480	16.004	2.971	5.445	8 971	3.840	5.442	6.090	4.423
D <sub>2</sub>	5.005	17.541	2-980	5.325	9.310	3-728	5.728	6.835	4.440
D <sub>3</sub>	4.866	15.410	3.059	4:929	8.753	3.612	5,868	7.128	4.218
D <sub>6</sub>	4.386	14 018	3.004	4.420	7.464	3.669	5.442	6.348	4:149
D,	4.047	13.649	2.742	5.051	9.671	3.099	5.619	6.173	4.279
D <sub>5</sub>	4.016	16.179	2.818	4.984	10.010	3.489	5.683	6.457	4.340

APPENDIX III.

Data used for graphical methods (see graphs).

Components	Group	Consumed from linseed	Total const paddy st linseed	umed from raw and l cake	Total di	gested
	~	cake	Mean	Error on méan	Mean	Error on mean
	0 lb.	grms. 0.0	grms. (2862·0)*	grms.	grms. (1751·0)*	grms.
Dry matter	1 "	400.0	4294.0	99:29	2044.0	78.94
Diy mabber	2 ,,	800.0	4736.0	99.46	2286.0	49.77
	3 "	1200.0	5163.0	40.44	2605.0	24.96
1	0 lb.	0.0	(3326)*	•••	(1687.0)*	***
Organic matter .<	1 "	367.3	3719.6	80-99	1963-2	74:32
organio matter	2 ,,	734.3	4117:3	· 79·17	2195.6	46.15
	3 "	1101.2	4509.3	32.48	2493.3	26.63
	0 lb.	0.0	(147.0)*	***	(13.0)*	• • •
Grude protein .	1 ,,	128.3	276.86	4.13	123.48	4.19
ande protein . 7	2 ,,	256.5	406.13	7.76	226.62	5.40
	3 "	384.7	535.74	6.83	340.96	8.74
	0 lb.	0.0	(45.0)*	***	. (19.5)*	***
Ether extract .	1 ,,	35.43	80.88	0.83	53.71	1.40
somer extract	2 ,,	70.82	116.56	0.89	87:46	1.37
	3 ,,	106.25	152.35	1.19	121.90	1.74
* ,	0 lb.	0.0	(1302-0)*	•••	(803.0)*	***
J J Cl	1 ,,	37.0	1348-2	34.66	817.84	36.04
Irude fibre≺	2 "	74.0	1395.6	59-28	813.10	87:30
h w	3 "	111.0	1440-9	11.61	838.01	64.19
, - ". "	0 lb.	0.0	(1830.0)*	•••	(845.0)*	***
Tra .	1 "	166.6	2013-65	- 43.43	968.21	36.61
Nitrogen-free ex-	2 ,,	333.3	2202-02	46.44	1046.93	37.65
	3 "	499.7	2380.36	19.05	1192:44	10.76

<sup>\*</sup>The figures in brackets have been directly read from the graphs.

## APPENDIX IV.

Detailed Calculations by the aid of Multiple Regression Equations.

The experiment was conducted with two unknowns, viz., paddy straw and linseed cake in three combinations, viz., 1 lb., 2 lb. and 3 lb., doses of cake.

There is no direct way by which we can establish whether there is any or no interaction taking place during the course of digestion with these two feeds. The same statement holds good for the progressive doses of cake as used in our experiment. The results worked out by the graphical method suggested that within the range of 1 lb., 2 lb., and 3 lb. combinations the inter-action at any rate is inappreciable. We may therefore neglect it for the purpose of our experiment, and as a first approximation, assume that the behaviour of cake and straw with respect to their individual digestibilities is independent of each other and also constant. The subsequent analysis of the results, as will be evident from the details in the text, also shows that this assumption is justifiable within the limits of sampling error in the case of the majority of the individual components of both the feeds.

Considering first the digestibilities of, say, crude protein (nitrogen) if  $x_1$  and  $x_2$  are the amounts consumed from straw and cake respectively, and if a and b represent the digestible fractions of  $x_1$  and  $x_2$  respectively, we obtain the linear relation

$$y = x_1 a + x_2 b \quad * \quad . \quad . \quad (1)$$

where y = the observed amount of total digestion from straw and cake.

If the equation gives a good fit, it should reasonably follow that the hypothesis holds good; and that the values of a and b thus obtained represent the nearest approach to the fractions actually digested from the respective shares of  $x_1$  and  $x_2$ .

In order that equation (1) can be made solvable by ordinary algebric process Gauss's method is employed whereby the following equations are obtained:

<sup>\*</sup>Since when  $\chi_1 = 0$  and  $\chi_2 = 0$  the amount digested is necessarily zero, therefore no constant has been added. From the observed values also, the value of c is found to be negligible in all cases.

Taking the case of protein (nitrogen), the summations of eighteen individual values of  $\kappa_1 y$ ,  $\kappa_2 y$ ,  $\kappa_1 \kappa_2 \kappa_1^2$ ,  $\kappa_2^2$ ,  $\kappa_2^2$ ,  $\kappa_2^2$ etc., are worked out as follows:--

-	×	Actually consumed	onsumed	Total				-	-	
	Bullocks	Paddy straw x <sub>1</sub>	Linseed ce ke x <sup>2</sup>	actually digested y.	$x_1x_2$	x <sub>1</sub> y	x_y	X <sub>1</sub> <sup>2</sup>	, K	er As
I lb. cake	$D_1$	24.19	19.60	19:14	474.12	463.00	375·14	585.16	384·16	366.34
*	$D_{\rm s}$	24.42	19.60	19-61	478-63	478 88	384.36	596.34	384.16	384.55
	$D_3$	25.25	96-07	21.53	529.24	543.63	451.27	637.56	439.32	463.24
-	D¢	25.32	20-96	21-21	530-71	537-04	444.56	641.10	430-32	449-86
	D,	22.14	21.02	20.08	465.38	444.57	422.08	\$1.06†	741.84	403.21
	$\mathbf{D}_{\mathbf{s}}$	21.31	21-02	16.98	448.15	361-84	356.92	79.79	441.84	28832
2 lb. cake	$D_1$	26.80	41.91	38-09	1123-19	1020-81	1596-35	718-24	1756-45	1450.85
	D <sub>z</sub>	25-27	16.17	37.50	1059-07	947-63	1571.63	638-57	1756.45	1406.25
	$D_3$	52:96	42.03	36-70	965-01	842.63	1542.50	527·16	1766-52	1346.89
	$D_6$	24.11	42.03	37-93	1013-34	914.49	1594.20	$581 \cdot 29$	1766.52	1438.68
	D,	21.61	39.15	34.38	846.03	742-95	1345.98	466.00	1532-72	1179-24
	$\mathbf{D}_{s}$	55.84	39-15	32.95	898.10	755.87	1289-99	526.54	1532-72	1085-70
3 lb. cake	$\mathbb{D}_1$	24.78	63-05	55-75	1562-38	1381-49	3515.04	614.05	3975-30	3108-06
	ã	54.00	63.05	53.09	1513-20	1274.16	: 347-32	576.00	3975-30	2818-55
	$D_s$	23-76	58.75	52.44	1395-90	1245.97	3080.85	564.54	3451.56	2749-95
	De	23.34	58-75	53.78	1371-23	1255-23	3159-58	544.76	3451.56	2892.29
	$D_7$	24.15	62.87	26.90	1518-31	1374.14	3577-30	583-29	3952.64	3237-61
	D°	24.94	62.87	55-35	1567-08	1380.43	3479.85	622.00	3952.64	3063.62
	Total .	:		663-41	17759-97	15964.76	31534.92	10367-94	35401-02	28133-51

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a = 0.0989854b = 0.8411323

The next step is to test the adequacy of fit for which the value of a and b as now obtained are applied on the 18 individual amounts actually consumed from paddy straw and linseed cake; and the expected values  $(y^1)$  representing the calculated amounts of consumption are worked out as follows:—

	Bullocks	Digested calcula	ted	Total digested amount (calculated)	Total digested amount (observed)	Difference (y¹—y)	$ \begin{pmatrix} \text{(Differ ence)}^2 \\ (y^1 - y)^2 \end{pmatrix} $
* * * * * * * * * * * * * * * * * * *		Straw x <sub>1</sub> a (x <sub>1</sub> ×'0959854)	Cake x <sub>2</sub> b (x <sub>2</sub> × 8411323)	y <sup>1</sup>	y		
1 lb.	$\mathbf{D_1}$	2.39	16.49	18.88	19·14	0.26	0.0676
cake.	$\mathbf{D_2}$	2.42	16.49	18-91	19.61	0.70	0.4900
	$D_{\mathfrak{g}}$	2.20	17-63	20.13	21.53	1:40	1-9600
	$D_6$	2.51	17.63	20.14	21.21	-1.07	1-14-19
	D <sub>7</sub>	2.19	17.68	19:87	20.08	-0.21	0.0141
	D <sub>8</sub>	2.11	17.68	19.79	16.98	+2.81	7.8961
2 lb.	$D_1$	2.65	35.25	37.90	38.09	-0.19	0.0361
cake.	$D_2$	2.50	35.25	37.75	37.50	+0.25	0.0625
,	$D_3$	2-27	35.35	37.62	36.70	-0.92	0.8464
	$D_{0}$	2 39	35 35	37.74	37.93	-0.19	0.0361
	D,	2.14	32.93	35.07	34.38	+0.69	0.4761
ere a por e	Dg	2.27	32.93	35.20	32.95	+2.25	5.0625
3 lb.	$D_1$	2.45	53.03	55.48	55.75	-0.27	0.0729
cake.	$D_2$	2.38	53.03	55-41	53.09	+2.32	5:3824
	$D_3$	2:35	49 42	51.77	52.44	-0.67	0.4489
	$D_6$	2.31	49.42	51.73	53.78	-2.05	4.2025
	D <sub>7</sub>	2.39	52.88	55.27	56-90	-1.63	2.6569
	$D_8$	2.47	52.88	55*35	55.35	0.0	0.0000
	Total .	•		663:41	663:41		30-8860

$$\frac{\varepsilon y^2}{n} = \frac{(663.41)^2}{18} = \frac{24450.72}{18}$$
Total variance 3682.79

From these data the statistical analysis of variance is obtained as follows:-

Analysis of variance.

	Degrees of freedom	Sum of squares	Mean sum of squares	Observed ratio of variance	Expected ratio of variance (Fisher's)
Multiple regression	2	3,651.90	1,825.95	886-39	6.351
Residual	15	30-89	2.06	• •••	1-1
Total .	17	3,682•79	обноступационня в протоцей гос	germania automorphis Methodologicale.	Proceedings of Artifest (

From the analysis of variance the co-relation is also worked out:—

It will be seen that in the case of crude protein (nitrogen) as worked out, the observed ratio of variance has provided an adequate fit and the co-relation has been exceptionally high (99.589 per cent). We can therefore take with sufficient reliability the values of a and b as the nearest approximation of actual digestibility.

The values of a and b for the other components, viz., dry matter, organic matter, etc., have all been worked out in the same way (table IX) and their analysis of variance are shown in Appendix V.

APPENDIX V.

Analysis of variance (Figures obtained by multiple regression equation).

Components		Degrees of freedom	Mean square	Observed ratio of variance	Expecte of var		Co-relation R
1	2	3	4	5	1% 6	5% 7	8
Dry matter	Regression .	2	542,871.58	·	• •	••	•
Dry matter .	Residual .	15	9,814.57	55•31 ∗	6-359	••	0.9384
The state of the s	***************************************						The same of the sa
Organ ic matter	Regression .	. 2	485,585.81		••	ž.	
Organ ic master	Residual .	15	8,656.57	56.09	6:359	* «	0.9392
-				Annya da maranta a cita da magani da cana da c		gywra fywriith aw tha ddyngy, mysyl ffelliddol i'r d	Anany de principal de l'agranda d'Agranda de Barela conti
Crude protein .	Regression .	2	1,825-95	*		••	•
Oraco protesti 1	Residual .	15	2.06	886:39	6-359	* •	0.9959
The state of the s		× .		-		***************************************	Managarah Managarah dan Jawa Jalang and
Ether extract .	Regression .	2	7,006-34	*	• •	•	
Forter express . 5	Residual .	15	83-90	83.41	6-359	••	0.9941
			* 1 ×		professional despite programme		
* Crude fibre .	Regression .	2	16,365-37		••	·	- • •
* Crude nore	Residual .	15	2,903-34	5 64	6.359	3.683	0.6551
		732.25			-	Plane Marie II revision and a second plane and plane	Manager with the control was an extension to the
Nitro	Regression .	2	88,8:3:67			10.00	-x 10 x 2
Nitrogen free (	Residual .	15	4,393-22	20.22	6:359		0.8541

<sup>\*</sup> Crude fibre has been dealt with separately. See text.

# DETERMINATION OF DIGESTIBILITY CO-EFFICIENTS

# APPENDIX VI.

Paddy straw and 1 lb., 2 lb., and 3 lb., linseed cake. Mean result of each combination.

Treatment		Dry matter	Organic matter	Crude protein	Ether extract	Crude	Nitrogen free
1	2	3	4	- 5	6 - ,	7	extract 8
	Paddy straw .	3.894.283	3,352.312	148 577	45.157	1,311-237	1,847.042
e de la constitución de la const	Linseed cake	400:000	367:279	128:279	35.426	36.967	166-607
1 lb. Combi- nation	Total .	4,294.283	<b>3,</b> 719·591	276.856	80.883	1,348-204	2,013.649
(Mean of 6)	Voided in facces.	2,250.675	1,756.353	i 53:375	27:171	530•365	1,045-142
	Digested .	2,043.608	1,963-238	123 481	53.712	817.839	968-207
-	% digested .	47.197	52.611	44.548	66.383	60.592	48.019
. (	Paddy straw .	3,936.800	3,382 999	149-677	45.738	1,321.697	1,869.846
	Linseed cake	799-667	734 252	256-4+9	70 823	73-902	<b>333·</b> 078
2 lb. Combi- nation	Total	4,736.467	4,117-251	406:1.6	116.561	1,395.559	2,202.924
(Mean of 6)	Voided in facces.	2,450·188	1,921.693	179:511	29·106	582:490	1,155-992
	Digested .	2,286.279	2,195.612	226.615	87.455	813-100	1,046.932
	% digested .	48-299	53:354	55.78	7 <b>5·</b> 035	58.254	47:397
	Paddy straw .	3,963.517	3,407.787	151.016	46.096	1,330.002	1,880.676
	Linseed cake	1,199-667	1,101.531	334.728	100.250	110-869	499-685
3 lb. Combi- nation	Total .	5,163.184	4,509:318	535.741	152:346	1,440.871	2,380-361
(Mean of 6)	Voided in facces.	2,557-693	2,016:001	194.785	30.433	602-865	1,187-919
	Digested .	2,605-491	2,493:317	340.959	121-916	838.006	1,192.442
	% digested .	50.470	55.298	63.661	80.324	58-153	49.983



# APPENDIX VII.

Digestibility co-efficient of paddy straw obtained by different methods and different authors.

Source.	Number ef trials	Different methods and authors	Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitro- gen free extract
1	2	3	4	5	i,	7	3	9
Market of the Control	18	Graphical method .	45.070	50.734	8.735	48-164	G1·650	46.172
	18	Regression equation method .	45.560	51-032	9:898	43.790	61-894	40.352
	6	By method of cake.	44.278	49:623	5.905	48 <b>·5</b> 98	60-692	45:295
Bengal	6	elimination with Henry cake.	42.058	47:390	-1.278	58-240	58 331	45.123
	6.	values. 3 lb. cake.	41.894	47.055	0.912	59:379	58:251	42.695
*	6	Paddy straw as sole feed	42-446	47:541	—13·322	41.456	59•729	41.695
Pusa old	12	F. J. Warth	47.000	57-100	4.4 -	60:200	72.000	44.100
Pusa new	***	12 27 4	52:370	58.840		41:340	72.960	41.950
Bangalore	9	.,, ,, .	53:540	60;610	11.170	43.410	70:430	57.710
Punjab (Kangra)	3	Lander and Dharmani .	42-730	48.590	19:160	46.670	60.720	42.100
American	6	Henry and Morrison .	***		22.000	23.000	59.000	46.000
Europeau .	4	Kellner .		47· <b>0</b> 00	45.000	47.000	57:000	32 000

## APPENDIX VIII.

Digestibility co-efficients of linseed cake by different methods and different authors.

Source	Number of trials	Different methods and authors	Dry matter	Organic matter	Crude protein	Either extract	Crude fibre	Nitro- gen free extract
( 1 )	2	8	4	5	6	7,.	ห	9
Bengal	18	Graphical method .	70.100	72.162	84.751	96.356	27:290	67:298
A September 1	18	Regression equation method .	65:150	67.069	84.113	95-600	7.709	61.845
American	3	Henry and Morrison .	79-000		89:000	89*000	57 <b>.00</b> 0	78.000
European	4	Kellner		79.000	86.000	92.000	32.000	78-000

# THE ETIOLOGY OF ENZOOTIC BOVINE HAEMATURIA, PART I.

BY

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(With Plates XIX-XXIX and 1 text-fig.)

### Introduction.

Among the animal diseases which have baffled elucidation notwithstanding the very considerable amount of scientific investigation that has been expended upon them in most countries of the world, chronic haematuria of cattle would appear to be one of the most outstanding. Since the articles by Anderson and Hubner were published in 1842. more and more attention has been devoted to the subject. Although various types of pathological processes affecting the urino-genital system can lead to the passage of unbroken red blood corpuscles in the urine, for that is what the term hacmaturia signifies, this disease entity has come to be recognised as a precise and clear cut clinical syndrome. Haematuria has been a serious scourge in cattle farms in many countries, and the first exhibition of blood in the urine leaves no other alternative for the owner than the disposal of the affected animals for slaughter as, from long experience, the futility of treatment has been recognised. The disease is known to occur in widely separated countries of the world including Australia, Austria Bulgaria, Belgium, British Columbia, England (Cornwall), France, Germany, India, Ireland, Italy, Kenya Colony, New Zealand, Oregon, the Pacific Islands, Washington, Scotland and Wales. The names under which the disease has been known in the various countries are as follows:-Red water, stallrot (stable red). Illawater Red water, Essential Haematuria, Haemorrhagic cystitis. Hematurie chronique des bovides, Haematuria vesicalis, malignant haematuria and Urocystis haemorrhagica, etc. In India the term haematuria seems to have been employed for the first time by Kristnasamiengar as late as 1896, but from the symptoms described by him it is evident that he was dealing with haemoglobinuria only. Credit is therefore due to Kerr for drawing attention in 1925 to the occurrence of true haematuria in Indian cattle at Kalimpong in Bengal and the disease has since then been investigated, also work has been carried out in the Kumaun Hills in the United Provinces, in the Nilgiris in the Madras Presidency and in the Kulu valley in the Punjab. In the last named province, it is reported that cases of haematuria in man occur in the same areas in which cattle are affected. It is said that in the Darjeeling District in Bengal, the people dread this disease more than rinderpest.

Many theories have been advanced to explain the causation of the disease, some of which are undoubtedly fantastic. Leaving aside such primitive theories as insufficient feeding (Anacher), poor feeding (Cruzel), periodical compression of the bladder by the rumen when it is filled unduly with voluminous food (Lienaux) stasis in the territory of the posterior vena cava (Hink), certain non-specific irritations mechanical, mycotic or toxic (Gotz), increasing precocity and diminishing resistance by crossing with a certain breed (Sinoir), the toxic effects of plants, including the young shoots of oak, ash, privet, hornbean, hazel, dog-berry, pine, fir, coniferae, [Law, 1901], ferns, sedges rushes hellobore, etc. (Galtier), and the trauma due to filaria or distoma (Lydtin), or that due to pentastomes (Burton and Cleland), coccidia (Arnold) or bacterial infection (Detroye) have been suggested. Among those who ascribe the disease to malignant neoplasms. Miyamoto [1927] seems to be outstanding, since he portrays the development of various types of neoplastic formation in regular sequence. This disease has also been considered to be an analogue of what is known in human medicine as acquired angiomata or internal sarcoids. The possibility of poisonous plants causing haematuria has been tested by several workers with a negative result in each case. For instance, Cleland [1911] experimented with Omalanthus populifolius (Euphorbiaceae). Indigofera australis and Goodia lotifolia (both Papilionaceae), and Hadwen employed in his tests extracts of dicentra (Bleeding heart), deergrass (Achlys triphylla), bracken (Pteris aquilina) and the extract of alder. This latter worker remarks that it is very improbable that one special plant causes the trouble, because vegetations in the various countries differ so much, and that a group of plants may thus be responsible rather than one particular species. In the Darjeeling district in Bengal the local people believe bamboo and fig leaves, which are common fodder there, to be the cause of the disease. Bamboo leaves (N. O. Gramineae Bambosa, arundinaea) are used by Indian villagers in cases of retention of the placenta in the cow apparently with a beneficial irritant action, simulating to a certain extent the effects of mild abortificients and Garudacher [1930] has reported the presence of hydrocyanic acid in bamboo leaf extract. Again Mustak Husain [1930] in the United Provinces incriminates tinpattia grass. From a knowledge of the toxicology of plants, however, one would expect a preponderance of constitutional symptoms, and in violent cases rapid death, if some irritant buds or shoots played a role; and in any ease the singular localisation of any toxic effects to the bladder exclusively would not seem probable.

In regard to the bacterial theory, the available evidence of most workers recorded in literature conforms to the experience of the author, which shows that attempts at bacteriological culture from the urine and internal organs invariably prove abortive in early cases. No bacteria have been seen in stained sections from internal organs in most cases, but diphtheroids and long chained streptococci have been seen and also recovered from the urine from clinical cases of old standing. Hadwen and others have failed to transmit the disease to healthy animals by cohabitation with clinical cases, by injecting haematuria urine into the bladder, by giving it by the mouth and by the injection of the blood and urine under the skin, and it may be noted that the former employed as many as 17 animals for the purpose. Durin and Unglas [1931] however consider the disease to be a coli-bacillosis, and they treated two cases with repeated doses of *B. coli* anti-virus but the results are not known.

Of the helminths, the finding by Ichikawa in 1922 of adult Schistosoma japonicum in the bladder tumours of two cases in Formosa indicated a possibility, which had the support of the existing knowledge of the bilharzial disease of the intestines and the bladder in man, and also of the experience of Sonsino [1876] who collected thirty Schistosome worms (S. crassa or S. bovis) from the blood of an ox, which presented intestinal lesions, and typical lesions of haematuria in the bladder. However Ichikawa failed to detect any bilharzial worms or ova in five other cases examined by him, and the experience in every other country has been similar. In this connection it may be noted that in Egypt Piot Bey [1918] recorded a case of bilharziosis as the cause of a perforating ulcer of the bladder of a calf, and in India Rangaswamy [1922] reported a case of haematuria in the Nilgiris, in the urine of which animal Schistosome ova are stated to have been seen. In the samples of urine from two other cases of the disease from the same locality, Parameswara Ayyar [1922] however records negative findings.

The position of the oxalic acid theory of Hadwen [1914, 1917] differs from the others mentioned above, since he claimed to have produced the disease experimentally with commercial oxalic acid by (1) injection of calcium oxalate crystals in aqueous emulsion into the bladder, and (2) by the oral administration of oxalic acid, in about 5 months and  $2\frac{1}{2}$  years respectively. The fact that he failed to obtain oxalic acid bearing plants in a sufficient amount to feed his animals had raised doubts regarding his contention, and in the absence of any clue for approaching the investigation of the disease, Datta [1931] pointed out the improbability of Hadwen's view and elaborated a metabolic theory suggesting an endogenous production of oxalic acid due to defective elimination. It seems reasonable to argue that the cattle suffering from the disease do not feed upon such unnatural forage in

excess as are known to contain much oxalic acid, e.g., rhubarb, spinach tomato apples, lettuce, grapes, cabbage, and the common Indian sorrel annul (Oxalis carinculata). In experimental oxalic acid poisoning, the localisation of the poison in the various organs has been studied by Servonat and Roubier [1911], and Chieri and Frohlich [1911] have shown that a nerve excitability results. Further the work of Craig and Kehoe [1921], Bull [1929] and Steyn [1933] finally disposes of the oxalic acid theory as untenable, contrary to the statement of the Vety. Bulletin [1931] to the opposite effect.

With the fall of this theory, however, the trend of opinion in most countries has been towards a chemical deficiency theory. During a tour in Australia, Theiler [1929] expressed himself thus. "What is required is a Laboratory in Mount Gambier itself and preferably on one of the affected farms. The remarkable coincidence of phosphorous and manganese deficiency in this area should not be lost sight of. A clue to further research may then be found". The Ottawa Report [1930] states "Previous investigations show that it (bovine haematuria) is not an infectious or contagious disease and point to the probability of a natritional disturbance and to mineral deficiency, hyperacidity, or to an unknown factor in the soil and vegetation as the cause of it". Again from the same country Fleming. Fowler and Clark [1930] report that the results of blood analysis to be within the normal range. In their experience two remedies have been found to have a beneficial effect on affected cattle: a change of drinking water from surface to artesian well water, and administration of ground coral rock. From analyses of the water and coral rock from affected areas certain possible causes of improvement have been suggested and field experiments outlined to test the possibility. In collaboration with Allardyce [1930] these workers again report that the blood estimation of 25 cattle, subjects of Haemaluria vesicalis, yielded normal amounts of cholesterol, sugar, nonprotein nitrogen, urea nitrogen, amino-acid nitrogen, creatine, creatinine, calcium, inorganic phosphorus, and chlorides. In the latest publication from Australia, Bull. Dickenson and Dann [1932] state that "the problem is not an easy one to solve, and it is possible that a successful method of prevention may be found before the actual cause of the disease is demonstrated ". In their opinion "it is possible that a deficiency of some dietary constituent is the essential cause of red water," and from the result of their urine analyses they go on to say that "there appears to be some possibility of a low protein intake and possibly a low sulphate intake. From the Calcutta School of Tropical Medicine, Ghosh [1933] records the results of chemical analysis upon the leaves of plants suspected of causing haematuria, e.g., Schima wallichii, Ficus nimoralis and Indian cherry, and since saponins were detected in the first two he states that feeding experiments with the leaves containing saponins are contemplated at the School. Again while this article was in preparation a claim has been put forward from the same city in the columns of a daily newspaper that "Maegregor and his assistants [1934] have been able to trace the cause of the disease to a protozoan parasite of the genus (?) Coccidium which, ordinarily, attacks the bowel producing intense anomia and dysentery", reviving thus the unsupported views of Arnold expressed in 1890 in Germany.

### DESCRIPTION OF THE DISEASE.

That this disease is an enzootic one has been observed since the earliest times. and some places have come to be known as bacmaturia localities. Attempts have been made by a process of elimination of factors, to trace any relation of the environment to the disease, and Hadwen, for one, has travelled extensively in America and Europe with this object. It has been stated that hacmaturia localities are sloping, mountainous land with a lack of cultivation and good natural forage, associated with a preponderance of ferns. In these areas the cattle have to roam in woods or partially cleared grounds, and the impermeable soils on which the forage grow generally, lack proper sunshine and are moist. As Law [1901] states, "it is the disease of woods and waste lands, of damp and undrained lands, of dense clays, of lands underlaid by clay or hard pan, of lands rich in vegetable humus, or vegetable moulds, the decomposition of which has been hastened by the application of quick lime", but "the disease has not been traced to any definite microbe or toxin". The drinking water in some of these localities is rich in mica or gravel, and the climate is generally cold. In Germany the disease has been described as occurring in stall-confined cattle, in France as occurring in low-lying districts, and again in others it is in the mountainous and sub-mountainous regions that the disease is seen. The disease has been stated to occur after a dry summer. during the winter, or in the spring. Moussu and Dollar [1905] however state that haematuria occurs just as frequently in the winter when the animals are housed, as in the spring when at pasture. Moussu states that this affection is very rare in young animals and is exceptional before the age of two and a half or three years. Both the sexes are attacked equally.

It is a noteworthy fact that irrespective of the country of origin concerned or of the incriminated cause, the clinical symptoms and histopathology of bovine haematuria have been more or less the same. In this disease the occurrence of any systemic disturbance of health is rare and in our experience no rise of temperature has been noticed prior to or following upon an attack of haematuria. The passing of blood being the only easily noticeable symptom in the living subject, the disease has been termed "essential haematuria" and the apparently contradictory term "symptomless haematuria" in human medicine signifies a similar type of condition.

The affected cases are seldom detected in their earliest stages. The condition does not attract notice until the blood corpuscles have attained a fairly appreciable proportion of the ejected urine, and have stained the urine distinctly red. The disease is not suspected in the earliest stages since the admixture with the urine of a quantity of blood serum, and microscopic amounts of blood cells, exuding from the commencing lesions in the bladder is easily overlooked. However, suspicions having been aroused, the disease is readily diagnosed by the microscopic examination of centrifuged urinary deposits or by the available chemical tests for blood. Frequent micturition, with blood passed at the end of or throughout the act, is seen, and mild symptoms of urinary colic may appear. When the loss of blood has reached a certain limit, the haemorrhagic urine tends to coagulate on the ground or even inside the bladder of the affected animal. The colour of the urine may be pale pink or bright red, depending upon the quantity of blood passed. The red corpuscles are usually completely intact, and there is nothing unusual about their size and shape. If there has been any retention of urine due to atony or due to an obstruction by clot formation at the urethral opening of the bladder, a crenation or actual breaking down of red cells takes place, rendering the urine coffee-coloured. As the disease advances, large-sized flakes of clots are also passed. Anaemic changes in the blood appear comparatively early, which persist and gradually become worse, and animal becomes extremely debilitated with the progress of time. When necessary, the condition of the bladder may be ascertained by a reetal exploration.

Haematuria is characterised by a slow and progressive course with frequent intermissions of variable duration extending from a few weeks to months. passage of blood may cease suddenly or by degrees, only to reappear in the same erratic manner. This process is repeated till the animal finally succumbs to extreme anacmia and debility, or to other complications such as severe internal haemorrhage into the bladder, hydronephrosis, uraemic poisoning or superadded infections of the urinogenital system and the alimentary tract. The occurrence of diarrhoea in the later stages of haematuria has been mentioned by a few workers, but no causal relationship between haematuria and the development of actual diarrhoea or the passing of semi-solid faeces mixed with mucus has been suspected. Although the brunt of the lethal effects of the causative principle of haematuria are borne by the urinary bladder, it is important to determine whether other internal organs share in the untoward effects, though not quite to the same extent as the bladder. In the uncomplicated early cases, there is apparently no evidence of kidney involvement, including oedema, rise of blood pressure, or the syndrome of renal colic, etc. Generally the disease lasts for months and years, and in the experience of Moussu [1905] an animal aged 28 years had been suffering for as many as 20 years, but no case of spontaneous recovery or successful treatment has yet been placed on record.

From this it appears that the pathological process in the bladder runs almost parallel to the recuperative mechanism of the animal's system. The disease has not been reported in young calves, and the cattle are stated to exhibit the symptoms after grazing for a number of years on one farm. History cards of all animals since their purchase from the surrounding hills are maintained regularly at this Institute. From a scrutiny of these, the time that elapses between purchase and the apparent commencement of haematuria symptoms appears to be short, and it appears possible therefore, that the majority of hill bulls at this Institute bring with them a pre-existing infection, rather than contract the disease here, though there does not appear to be any reason why such an immunity of this place should exist. In the author's experience in 1928-29, cases had been removed from the hills to Calcutta, but a change of locality did not produce any substantial alleviation of the disease. It has been observed at Muktesar that a number of cases invariably exhibit exacerbations of haematuria with snow-falls, and a gradual and continued decrease of the bodily temperature indicates a real danger signal.

## MACROSCOPIC LESIONS.

On post-mortem examination naked eye lesions of significance are not usually discernible in any organ excepting the bladder, but occasionally the kidney or the ureters may be abnormal. The bladder may be empty or filled with haemorrhagic urine, which is partially or completely clotted. It has been suggested that it is only in the basal and ventral part of the bladder that the initial lesions are formed, but when the gravitational precipitation of the causal factor can be climinated, such a suggestion has no significance. On eversion of the organ, variable lesions are encountered. The early lesions appear as small patches of congestion, and red dots of haemorrhage up to the size of a lentil, associated with a slight thickening of the mucous membranes are seen. The altered mucous membrane is raised above the surrounding areas as small round yellow areas enclosed by a ring of acute congestion. Localised gelatinous infiltration of the submucosa is seen, which is followed by the sloughing of the overlying mucous membrane, giving rise to small ulcers, which form the seats of continuous blood-letting until a clot forms. The ulcers are discrete, and, when confluent, become broader or more elongated and present raised edges, assuming a button or crater-like appearance. Since the opportunities of examining genuine early cases are not readily obtainable, the apparent first appearance of haematuria being possibly an intermittent attack only, the naked eye lesions are seldom so simple as the above. On the contrary, one may find "varieosities as large as hemp seeds, proliferations as large as nuts", rounded or cauliflower-like, rough raised red ridges, eroded bleeding patches, and submucous haemorrhages, sessile or pediculated pyriform mucoid eysts, and considerable thickening of the bladder wall

(Plate XIX, Fig. 1). Cicatrices are not very evident. Only localised areas of the bladder may be involved or the lesions may be diffused throughout. In cases of old standing, considerable ulceration has been detected at post mortem, although blood was not being passed immediately prior to death. The co-existence of such a variety and gradation of lesions, from minor changes to exhuberant growths, shows that the lesions develop separately and continously, and not simultaneously, explaining the remarkably persistent and intermittent character of haematuria.

In these studies mucoid cysts have been met with on the surface of the kidney, and minute haemorrhages have been seen in this and other organs. Hydronephrosis or cystic kidneys seen in haematuria cases seem to be connected with the bladder lesions. The intestines, particularly the caecum and the proximal end of the rectum of haematuria subjects have shown very minute sub-epithelial haemorrhages or millet seed and pin-head sized round ulcers with raised edges and deep necrotic core, but their significance is not known at present. Plate XIX, Fig. 2 shows polypoid growths in the duodenum from a case of haematuria.

## CLINICAL AND CHEMICAL STUDIES.

Although in the course of routine post-morten examinations at Muktesar a few cases of the typical bladder lesions of haematuria were undoubtedly detected earlier, it was only after the first report by Kerr [1925] that the disease received attention, The disease has figured in several Annual Reports of this Institute since that date. In the earlier investigations, arrangements were made to collect full clinical details regarding haematuria, including weekly body weights, daily temperatures, etc. A method was improvised to approximate the proportion of the admixture of blood cells and clots to a fixed amount of urine passed. Blood smears were examined regularly, samples of urine from each fresh case and post-mortem material were subjected to culture examination. Experimental treatment with trypan blue, and the benziding test for the presence of haemolysed blood in samples of the uring, from which the centrifuged deposits had been removed, were carried out; and thus the possibility of bovine piroplasmosis was first eliminated. By centrifugalising the urine and faeces from uncomplicated haematuria cases, and by means of the sugar floatation technique, repeated examinations were made and the possibility of coccidia was consistently negatived. Repeated attempts to culture any protozoan organism from urinary sediments by the use of 5 per cent. potassium dichromate solution and of hay infusions have given negative results. As oportunities occurred, careful post-mortem examinations were made in order to discover if the lesions in any other organs excepting those of the urinogenital system, could be correlated with the passage of blood in the urine. At that time the probability appeared to be that "the cause of the disease was an irritant chemical substance elaborated from the excretory products in the urine while the urine was stationary in the bladder." Investigation of the disease on the above lines continued, but it was increasingly felt that unless more information on some specific factors relating to the extravasation of blood from the bladder was available, the study of the disease was not likely to be productive of anything but negative data, and a critical examination of all the causes suggested in literature was made. As has already been mentioned earlier in this article, Hadwen's was the only work which claimed to have reproduced the disease experimentally, but, even so, his views appeared to be improbable. Therefore from theoretical considerations of available biochemical knowledge [Datta, 1931] it was considered advisable to determine whether there was in the system, any stagnancy of oxalic or other allied acid, such as parabanic acid (Oxalylurea) of metabolic origin, and also whether the deficiency of calcium or other metallic constituents of normal body tissues, or any haemophilia, existed, which could explain the tendency to the extravasation of blood seem in the living animal.

Since, in a few cases which had died of haematuria, the blood in the body was found to be still in a fluid condition, and with little tendency to clot, attempts were made to test the coagulability of blood. Various methods of determining the coagulation time of blood were tried, and after preliminary trials with drops of blood on glass slides, also in corked and open phials, had been made, the Loop method of Inchley [1921] was chosen as the most suitable working method. The serum calcium of a number of healthy and affected hill bulls was studied. Calcium was administered in various forms to affected cases, since it was desired to raise the calcium content of the blood in the hope of counteracting haemophilia, if such was present. Curiously, the same average clotting time of about 11 minutes was observed in all animals, whether in the active or quiescent stage with regard to the passage of bloody urine. The administration, morning and evening, of calcium lactate in the feed, and of calcium chloride solution intravenously, did not produce any marked difference in the coagulability, of the blood in affected cases. The clotting time in one case, however, rose to 18 minutes a short time prior to death. Similarly when the serum calcium was estimated in those haematuria subjects which were receiving calcium therapy, for purposes of comparison with those that were not, the calcium value showed only a small increase, but the phosphate value in the treated subject was decidedly on the increase. Another noticeable fact in these experiments was that, even during the period when as much as 5 per cent, of the urine passed by the animal consisted of red corpuscles, the animal was seen to be putting on weight consistently, and this may be ascribed to the benefits of calcium therapy. The result of chemical analy350 THE INDIAN JOURNAL OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY [ IV, 1v.

sis on an average sample of haematuria urine, from an untreated case is given below. The figures are per 100 c.c. of serum.

Amount of PO<sub>4</sub> (phosphate) = 0.0148 gm. ,, ,, Calcium = 0.024 gm. ,, ,, Albumin = 0.060 gm.

Urea was not estimated. In order to ascertain whether there was any hyperacidity of serum in affected cases, the PH values of some healthy and eight haematuria subjects were determined colorimetrically, with the result that the former gave 7.6 and the latter 7.5. There was therefore no marked discrepancy, but whether a large proportion of neutral salts of acids were present was not determined.

With regard to the urine itself, its specific gravity, reaction and composition were also studied. In our experience, the specific gravity of haematuria urine varied from 1020 to 1035 in different individuals, and from the observations made on several animals, the rise and fall in the specific gravity did not appear to bear any relation to the percentage of blood present in the urine at different intervals. The reaction of the urine in each case was found to be alkaline to litmus, phenolred, and brom-thymol blue. Samples of urine from three cases were examined for the presence of oxalates, sugar, etc., with negative results. As was to be expected, albumen was present.

# GENESIS OF THE DEFINITE CLUE.

The etiological researches upon haematuria took a definitely hopeful turn on the 7th of March, 1932, when the author discovered some very interesting 'bodies' in the course of histological studies upon specimens of preserved bladder and kidney tissues, collected from local haematuria cases (Plate XXIV, Fig. 2). When, some peculiar spherical bodies were detected in the urine of all the six clinical cases of the disease then at Muktesar, the securing of 'data of considerable value and promise' was mentioned in the Annual Report of this Institute for 1931-32 (p. 15). They appeared to differ from body cells. They were globular bodies whose nuclei could be discerned with difficulty in unstained wet smears; these bodies appeared to be absent from the urine from healthy cases, but were present in the quiescent stages of haematuria. To ascertain if these bodies were present in samples of haematuria urine from other provinces in India, enquiries were made from the Heads of Veterinary Departments in the provinces, where the disease occurs—viz., Bengal, Madras and the Punjab. On the arrival of samples

from the provinces the same obscure structures were detected, and the possibility of a Schistosome origin was suggested. In the Annual Report for 1932-1933 (p. 15) the failure to obtain confirmation of the above suggestion has been mentioned, but the observation of 'a definite host reaction 'against the 'bodies' left no doubt that they were parasitic and foreign (Plate XXIX, Fig. 1). In size they were larger than the usual body cells, and they definitely had a greater depth of focus. Since an occasional 'body' appeared to have engulfed rare red corpuscles, or to possess a vacuole they were described in the above Report as 'peculiar macrophage-like cells,' and the possibility that these were Entamocha was pursued by means of the cultural technique of Boeck and Drbohlav [1925]. The first results have already been mentioned in the Annual Report of 1932-33. Before giving a preliminary description of the causative parasite, which appears to be responsible for this disease, it is essential that the minute histology of the condition be first described in the light of fresh knowledge, since the current views are faulty in some respects.

## PATHOLOGICAL HISTOLOGY.

The Urinary Bladder. One would not expect to detect a bovine haematuria at its very commencement with a view to studying the pathological process in its earliest stage. Young and old lesions are, however, present in most cases and these may be utilized for histopathological studies. The disease is primarily an affection of the submucous layer, inflamatory exudation into which leads to necrosis and consequent ulceration of the mucous membrane. The bladder is intensely congested, and the blood vessels supplying the mucous and submucous layers are greatly dilated (Plate XX, Fig. 2). The capillary vessels are often ruptured, and extravasation of blood around them is to be seen. In some cases however the whole of the bladder wall is extensively infiltrated with blood and its pigments. The vascular endothelium is damaged to a certain extent, and the exudation of serum to the perivascular regions leads to a pushing apart of the surrounding connective tissue. Sometimes oedema is very pronounced, and is easily discernible immediately below the muscularis mucosa and in the strands of tissue, which intervene between muscle fibres.

The diversity of the pathological processes and the extent of their severity seen in each case, depend upon the degree of the parasitic infestation, rate of division of the parasites, and upon whether the lethal effects are repeated over a length of time. The susceptibility of the affected bovine or its bodily resistance must also be a factor of importance in the pathogenesis.

As a result of the inflammatory exudation, the epithelial lining of the bladder is swellen and elevated, the inflammatory products separating the mucosa from the thickened submucous layer. The extravasated blood and exuda-

tion are partly absorbed or removed. When the amount of this is more than can be conveniently disposed of, degenerative changes in the overlying epithelium leads to the formation of ulcers by sloughing. Blood is then easily lost from the widely-open sub-epithelial vessels, and haematuria manifests itself. If the parasitic incitants have been removed with the slough and haemorrhage, and a fresh batch of parasites are not present in the immediate vicinity, organisation of the broken epithelium commences. Clots are first fermed on the raw surfaces and local capillary thrombosis may take place. Extravasation of blood ceases and haematuria disappears for the time being. The seat of attempted cicatrisation remains a weakened spot, and further progress of the recuperative process may be undermined by the intervention of a fresh batch of the parasites. Even if the obliterating clots or thrombi were to persist and the lesion were to be completely resolved, fresh lesions appear elsewhere with the reappearance of haematuria. The disease thus obtains a foothold, and tends to become chronic, and the diverse types of lesions. known to characterise the disease, manifest themselves. The lining epithelium of the bladder may still appear to be in tact in the major part, but depressed ulcers of small size with raised edges, or even coalesced patches of ulceration are to be seen. Generally these areas of ulceration are superficial and do not extend much below the submucosal layer, but deep ulceration may at times be encountered as well. Immediately under the epithelium and extending only a relatively short distance into the deeper tissues are blood cavities and channels of various sizes and shapes (Plate XXII, Fig. 1). The connective tissue stroma, which supports these extremely vascular or haemorrhoidal areas, appears to have undergone some hyperplastic changes. Apart from the several scattered points where the denuded areas of mucous membrane are in the process of cicatrisation, one notices a proliferation and thickening of the epithelial lining. Obviously the series of changes through which the affected mucosa passes must depend upon the severity of the traumatic injury or irritation, and also upon the chronicity of the case. In such areas of the mucosa, where the traumatic influences have not directly operated, or where they have not been unusally severe, the intact epithelium has to adapt itself to the mild irritations to which it is being continually subjected from time to time. The bladder tissues attempt to adjust themselves to the changed circumstances by the production of branching cauliflower-like tassels of soft papillomatous growths. The character of the epithelium of the bladder is maintained over these growths, and branches of blood vessels are carried into each prolongation. A few pyriform mucoid cysts are formed since the escape of the secretion of the glands of the bladder is obstructed (Plate XIX, Fig. 1).

The fundamental lesions of the disease are however formed in the small and large-sized vessels, which show definite reactions within and around themselves, irrespective of their situation in the thickness of the bladder wall. The submu-

cosal vessels are the first to exhibit the lesions of the disease, and like inflammatory conditions in other diseases, new vessels are produced in this situation. The majority of the vessels are more or less dilated, and this dilatation is accentuated in some places more than in others. The endothelium of individual vessels shows proliferation and desquamation, and the lumen contains some of the active forms of the parasite. If thrombosis of a vessel occurs, and this is aided by the parasites being in the sluggish stage, the arrest of the parasites takes place (Plate XXIX, Fig. 1). A process of fibrous encapsulation from the periphery inwards takes place, and an attempt at the digestion of individual parasites is made by the body cells (Plate XXIV, Fig. 2). The affected vessels undergo varying degrees of endarteritis obliterans. Some vessels are actually obliterated, and in others partially sclerosed, a few parasites may still be seen to persist (Plate XXIX, Fig. 1). The localisation of parasites in altered vessels has been seen at all depths to the subscrosa.

In the continuity of the bladder epithelium, one often notices several solid nests of epithelium, which are generally disposed in the form of rounded adenomatous cysts but apparently without any central lumen. Various stages of development and degeneration of these epithelial nodes are seen (Plate XXIII, Fig. 1). As these nodes enlarge, their central zone shows some thinning, while a few prominent nucleated "bodies" are clearly seen. With further development of these nodes, the nucleated "bodies" show more structural differentiation, and when the evidence of rare parasitic encystment is observed one has no hesitation in recognising the initially undifferentiated nucleated "bodies" as the forerunner of the larger sized parasites or of their encysted fellows (Plate XXIII, Fig. 2). The collection of several layered epithelium in nodes appears to be more frequent than the normally existent mucus-secreting glands of the bladder, and evidence seems to be available to show that a number of these nodes may originate from the bladder epithelium (Plate XXII. Fig. 1). Since a number of such nodes are also seen to be present in the depth of the bladder, there need be little doubt that they can originate from blood vessels. Further it seems possible that the papilliform processes of the mucosa, as they grow, may close and form tubular adenomatous structures. Degenerative changes in the nodes extend from the centre towards the periphery, and in the advanced stages all the epithelial cells may be destroyed, giving place to a collection of an increasing number of parasites. The enclosing membrane then bursts, and the parasites are liberated into the urine (Plate XXV, Fig. 1).

In the most oldstanding cases, the blood vessels at all depths of the bladder wall show an extreme proliferation of the endothelium and appear as only collections of epithelial cells, bearing little or no resemblance to the original blood vessels. In some cases these epithelial cells exhibit a definite infiltrative type of extension, and show unmistakable tendency to simulate malignancy (Plate XXVIII, Figs. 1

& 2). So-called "bird's nest bodies," of epithelioma are however never to be seen, and real metastasis into other organs has not been found.

Another abnormality present in the sections consists of scattered minute collections of cellular infiltration, immediately beneath the epithelium and also around the ulcerated or haemorrhagic lesions. The cells are generally of the mononuclear type, consisting of young fibroblasts, lymphocytes, and endothelial cells with an admixture of so-called plasma cells. The most interesting picture is however to be seen below the clots and ulcers. The central core of an ulcer consists of nectrotic material with cellular debris and fibrin. Around the degenerative core is a cellular reaction zone, the margins of which contain the invading parasites. Plate XXI, Fig. 1 represents an active haemorrhagic ulcer involving the mucosa and submucosal layer. Just below the haemorrhagic ulceration a colony of a number of parasites, enclosed in a capsule and lying in a pool of acute extravasation and with little attachment to the supporting bladder tissue around it. is noticed (Plate XXI, Fig. 2). This picture leaves little doubt about the parasitic etiology.

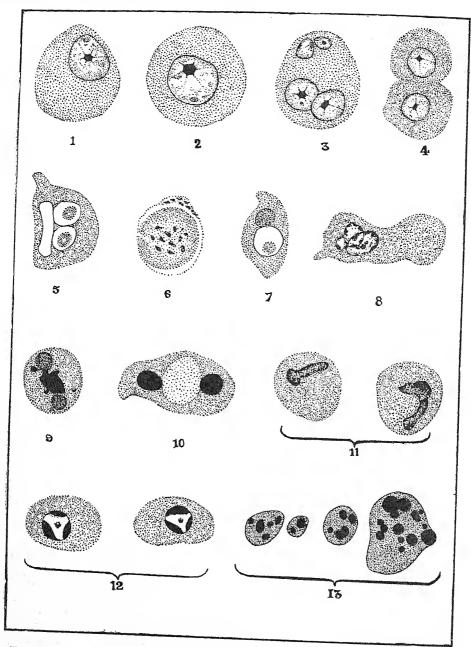
In regard to the parasites, it has been stated that they are found in the epithelial nodes on the lining membrane of the bladder, and in the lumen of blood vessels which are more or less altered. The occurrence of the parasites in the reaction zone around the degenerated areas of the submucosa has also been mentioned. Further the parasites may be found considerable distance beyond the initial reaction zone, and may even be seen making their way along the fibrous septa between the muscle bundles. The parasites may be numerous in the active lesions, but occasionally it may be difficult to find them. In the chronic cases, the muscular layer and the subserosa may become involved. In the more acute cases however, the parasites are situated exclusively in the submucosa. In acute attacks and early lesions, the parasites are easily recognised but in chronic cases this may need some perseverance.

Kidney. The parenchyma of this organ shows haemorrhagic areas, and cloudy swelling in places. The glomeruli show varying degrees of dilation and degeneration and the epithelial cells of the tubules are unrecognisable. The parasites have been detected in this organ also. (Plate XXVI, Fig. 1.)

### THE PARASITE.

The parasite varies in shape and size, and can be seen both in a free and an encysted stage. Its cytoplasm appears to be sharply defined, having an alveolar or reticulated appearance. Inclusion bodies and ingested red corpuscles have been seen, but bacteria seem to be singularly absent amongst the ingested material. The parasite possesses definite histolytic powers, and appears to grow by the absorption





Free hand drawing illustrating the characteristic features of the parasite, as seen in Sections.

Figures 1, 2, 3 and 4. Parasites showing cytoplasm, nucleus, karyosome and linin net-work.

Figure 3. Shows nuclear division into four, and Figure 4 shows a parasite divided into two. Figure 5.

Shows rod-shaped chromatoid, body and glycogen vacuoles. Figure 6. Shows a fragmented nucleus.

Figure 7.
Figure 8.
Figure 9. Shows a nucleus and glycogen vacuole. Dividing nucleus and irregular shaped parasite. Divided nucleus with an irregular mass of glycogen.

Figure 10. Two ingested red cells and glycogen vacuole. Figure 11. Dividing nuclei.

Figure 12. Massing of chromatin into three irregular clumps.
Figure 13. Inclusion bodies in parasites. Section stained with iron hæmatoxylin.

of food material through its body surface. Granules of blood pigment have been seen in its cytoplasm, and the ingested crythrocytes appear to have undergone some shrinkage. In the smallest sizes, the cytoplasm is clear and shows no ingested bodies. Contractile vacuoles do not appear to be present, but so-called chromatoid bodies and glycogen vacuoles are occasionally found. Microphotographs of collections of the parasite as seen in histological sections are appended hereto. A diagrammatic representation of the characteristic features of the individual parasites is given (Plate XXIX (a)). In shape the parasites are usually spheroidal (Plate XXVII, Fig. 2), but irregular shapes are also seen. Occasional prolongations of the cytoplasm (Plate XXV. Fig. 2), are met with, and these are probably pseudopodia. Some parasites are elongate, as seen in (Plate XXI, Fig. 2). No suggestion of the presence of flagella or cilia have been detected. Perhaps the spheroidal shape indicates a state of rest, and the clongate shape that of activity. Differentiation into endo-and ectoplasm is not discernible in sections, but the sharp outline of the parasite obviously indicates such a hyaline ectoplasmic structure. The nucleus of the parasite is the most outstanding feature. It is vesicular and round, and possesses a centrally situated dot, the karyosome. The nuclear membrane is very definite, and presents on its inner surface a series of bead-like small granules of chromatin arranged uniformly. The clumping of the peripheral chromatin beads into three irregular masses on the internal surface of the nuclear membrane has been seen in one or two exceptional parasites (Plate XXIX (a), Fig. 12). From the karyosome a network, presumably of linin, is seen to radiate to the nuclear membrane. On careful examination, the nuclear membrane is found to be an even circle of highly refractive material of some depth of focus. The encysted and free forms of the parasite, encountered in sections from half a dozen cases of haematuria have been measured in microns as follows:-

F	Cysts.	
41·3 × 12·0	44·0×18·7	
33·3 × 13·3	39·3 × 14·7	14·4×9·4
34·7 × 14·7	48·0 × 12·0	28·3 × 28·3
29·3 × 17·3	53·3 × 10·7	24·3 × 17·5
34·7 × 14·7	41·3 × 17·3	31·7×22·9

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-		Free forms.			Cysts.
	29·3 × 13·3	-	36·0 × 12·0		28°3×16°9
	24°0×10°7		41·3 × 21·3		
	16·7 × 9·3		36·0 × 17·3	-	
	21·3 × 10·7		40°0 × 13°3		
	13·3 × 9·3		40°0 × 10°7		
	22.7 × 16.0		36·7 × 32·0	Autority in the control of the contr	
	33°3 × 29°3		48.0 × 32.0		
	$44^{\circ}0\times25^{\circ}3$		46•7 × 20·0		
**	24°3 × 21°4	* *************************************	44.0 × 16.0		
	30·0 × 16·4	\$	40.0 × 16.0		
	17.5 × 17.0	9	56.0 × 40.0		
* 4	28·5 × 30·0		30·7 × 25·3		
	15·7 × 12·8		25·3 × 22·7	at to and displaying the second	
	34·7 × 14·7		34·7 × 30·7	-	
	33·3 × 13·3	*	30·7 × 25·3		
	37•3 × 9•3		18.6 × 16.4		
	32·0 × 20·0	2 2	20.0 × 20.0		
	60°0 × 14°7				

The cysts are rare in sections and are found lying somewhat separated from the host's tissues. A thick double-contoured capsule with the evidence of one, two or

three nuclei has been seen (Plate XXIII, Fig. 2 and Plate XXV, Fig. 1). The size of the parasites is important in order to distinguish them from body cells, such as, epithelial cells, pus cells, macrophages or other bloated cells. The size of the double contoured cyst is helpful in the identification of the parasite. It is in the smallest stages of the parasite that one has to be particularly careful in distinguishing them from bloated or wandering cells.

In the nondividing stage, the nucleus of the parasite varies from 5.7 to 7.3 microns. Nuclear division takes place by a process of binary fission, and the maximum of up to four nuclei has been seen in dividing individuals.

The parasite is best studied under the 1-6th objective with frequent use of the 1-12th oil immersion lens. It has also been studied in the fresh condition in wet smears, prepared from the clear and bloody urine obtained from hæmaturia cases. Since urinary sediments contain a variety of cells one has to examine the former from a large number of healthy cattle, before forming conclusions regarding the structures encountered in the urine of haematuria subjects. Parasites of spherical shape, which may show an ectoplasm, and several times larger than body cells, with inclusion bodies and a hazy nucleus in unstained preparations, are to be seen in haematuria urine. Highly vacuolated and granular bodies probably represent degenerated parasites. Spherical double-contoured encysted parasites, the largest measuring about 35.4×30.8 microns, are to be found. Rarely a small pore-like structure has been discerned in the cyst wall. On the warm stage parasites with digit-like or blunt dome-shaped pseudopodium have been seen. Under the microscope the parasites have not shown any attempt to cross the field nor have any simultaneous retraction and protrusion of processes been seen. They would thus appear to be in a somewhat moribund state in the urine.

From the above description, it will be appreciated that the parasite has no features of the phylla ciliophora, mastigophora and sporozoa, but would appear to belong to phylum Rhizopoda. The parasite is characterised by a nucleus with a small spherical karyosome and a peripheral layer of fine chromatin beads, justifying for it a place in the genus Entamoeba. The size of the free forms and the cyst is much larger than that of all the known species of Entamoeba, and the host is a bovine. Definite histolytic powers are possessed. In view of these considerations, and the fact he has received considerable help from his wife Mrs. Kamala Datta the author suggests the designation E. Kamala, n. sp. for the large protozoan parasite causing bovine haematuria.

### DISCUSSION.

It will be of interest now to consider whether the course and nature of bovine haematuria, as also its general morbid anatomy and histology, are in conformity with what is known about natural and experimental amoebiases.



In both amoebiases and bovine haematuria, one notices a progressively, mild chronic course with periods of quiescence between exacerbations, and the occurrence of lesions in apparently healthy cases. Moussu's [1905] case, already mentioned, was known to suffer from attacks of haematuria for 20 years, and similar remarkably persistent cases of amoebiasis in man have been encountered. With regard to the possibility of haematuria and the bladder lesions, as encountered in cattle, being due to amoebiasis, considerable support is available in relevant literature. Of the numerous cases of amoebic cystitis associated with haematuria which have been met with in the diverse races of the world, authorities in protozoology are prepared to accept notably few cases as genuine amoebic infections, such as Walton's [1915], Fischer's [1914], and Petzetakis' [1923]. Wirthin's case of amoebic infection of the testis and epididymis has also been accepted. From the few cases of amoebiases recounted below it will be seen that the maintenance of an over-critical attitude from theoretical considerations of protozoology without reference to the nature of the clinical disease or of its lesions, can no longer be justified.

Commencing with such interesting cases of amoebiases in large animals, which have not found mention in any of the standard books or in the literature upon Amoebiases, one finds that Benome [1895]\* published an article on "Uber parasitare Ictero-haematurie der Schafe. Beitrage Zum Studien der Amoebosporidien" and Lehman [1912] dealt with cases of amoebiases in the horse, cattle and sheep. Lehman showed that in these animals amoebae induce papillomata and other neoplastic growths. A comparison of the picture of the warty growths in sheep found by him with Plate XIX, Fig. 2, of this article is very suggestive. In discussing Lehman's findings, an Indian Veterinarian, Valladares [1913] makes the remark that "it would be worthwhile keeping a look out with a view to ascertaining whether the amoebae as the cause of disease in the domesticated animals exist in India," and the pertinence of that remark has been illustrated in the present investigation and also in the records by Ware [1916] and Boyd [1931] upon amoebiasis of dogs in the Madras Presidency.

Turning now to the pathology of human amoebiases, Brown [1910] states: "Not only is the submucosa actively inflamed and infiltrated but the connective tissue fibres proliferate, and minute buttons of adenoid tissue push up from below and appear as cluster of wart-like buds on the surface of the mucous membrane.

\* \* \* \* None of the morbid changes of amoebic dysentery are more distinctive of the disease than the exudation of pus from minute orifices of mammillated growths on the mucosa. \* \* Many of the epithelial cells are replaced by mucous cysts." Jurgens found mucoid cysts in his case of chronic

<sup>\*</sup> Original not consulted.

cystitis. A resume of the early cases where amoebae were found in human urine has been given by Dobell [1919], and Fiessinger and Parturier [1926] have reviewed the subject since. In a demonstration before the Royal Society of Tropical Medicine and Hygiene, Yorke and Adams [1928] described an interesting case of 'colloid carcinoma' of the splenic flexure of the large intestine, in the lesions of which the presence of great numbers of Entamoeba histolytica was proved. Again in an article on "Amoebic granulomas of the large bowel and their clinical resemblance to carcinoma," Gunn and Howard [1931] describe their own experience and draw pointed attention to the occurrence of localised tumours of amoebic origin, which present the clinical and gross pathological picture of carcinoma. They proceed to cite the previous experience of Desjardins, who coined the term pseudocancer for cases of amoebiasis resembling cancer but responding to medicinal treatment. It will not be out of place here to quote Knowles [1928] who remarks that "it is just possible that amoebic infection with its consequent irritation may be one of the factors concerned in the production of primary carcinoma of the liver of man in India.

Again in the case of experimentally infected guinea pigs the occurrence of lesions resembling neoplasms is definitely known, though to Dobell and O'Connor [1921] the lesions seen appear to be peculiar.

Having seen how closely the morbid anatomy of bovine haematuria conforms to the available knowledge on amoebiasis of man and animals, one may turn to the probable method by which amoebae gain access to the urinary bladder. In cases of human amoebic cystitis, the question is still obscure. From the histological features of bovine haematuria, the blood stream appears to be the chosen route. Secondary infection of the brain, liver and other organs have been encountered in man, and the parasites have been detected in blood vessels in some of these cases, but the possibility of the blood circulation playing an important role in the generalisation of amoebic infection does not appear to have been considered at all seriously. From the studies in the present cattle disease, the need of this possibility being investigated is emphasised.

### Conclusion.

Definite evidence has been put forward in this article to show that the hitherto obscure enzootic bovine haematuria is a parasitic disease, caused by a large protozoan parasite. From the characters of the parasite, it appears to belong to the phylum *Rhizopoda* resembling *Entamoeba histolytica* but would appear to be a new species, for which the designation *Entamoeba Kamala n. sp.* is proposed.

Results of further work in connection with experimental transmission, cure etc., which are now in progress will be published in due course.

Acknowledgment. Thanks are due to Mr. Sundar Rao, Artist, for preparing the photographic plates and to Mr. Ahmed Bux, Assistant Artist, for making the drawings. The author is indebted to Mr. Krishna Iyer, G.M.V.C., for measuring the parasites and their cysts from time to time.

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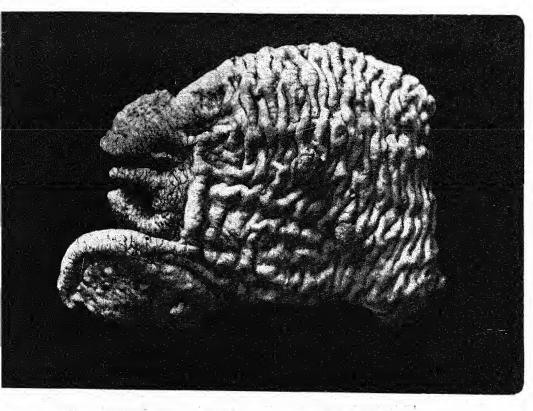


Fig. 2. Two warty growths in the duodenum, a few inches from the pyloric end of the abomasum. Microscopically, sections showed resemblance to bladder-growths and the presence of rare parasites.

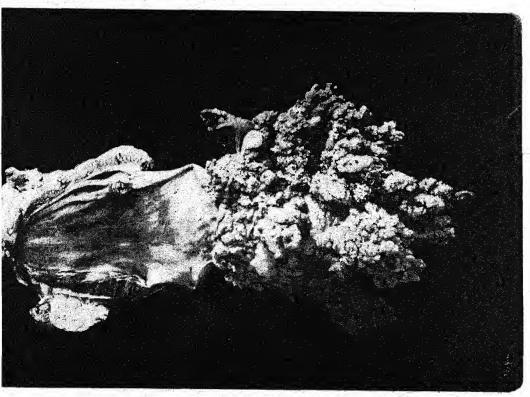


Fig. 1. Inside of the urinary bladder shows extensive cauliflower-like growths and two pyriform mucoid cysts.



Fig.  $1\times 56$ . Bladder, Junction of healthy and desquamating mucosa. Surface is hæmorrhagic, undergoing coagulation necrosis under the action of developing parasites situated immediately subjacent to the degenerating mucosa,



Fig.  $2 \times 40$ . Shows extreme dilation and engagement of the capillaries supplying the mucosa and



Fig. 1 imes 244. Early lesion, reaching nearly to the muscular layer, resembling a 'typical ulcer of Harris' of human amebiasis.

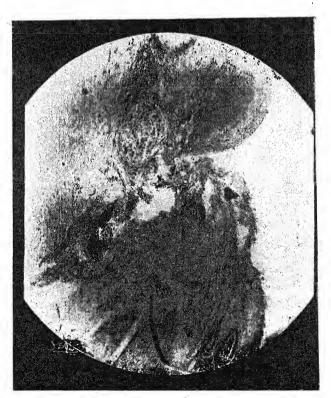


Fig.  $2\times56$  shows the nidus of the disease in the submucesa, represented here by a colony of parasites in a thrombosed and bursting vessel, with secondary changes in the mucesa.

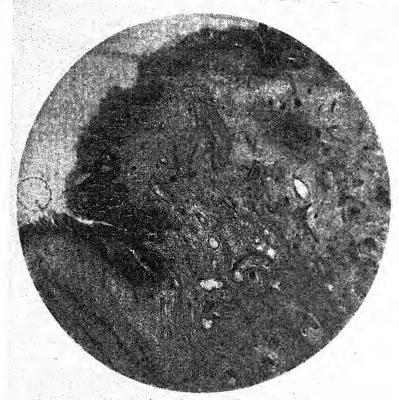


Fig. 1. Bladder mucosa shows considerable proliferation in the centre of the field, it is disposed into an adenoma-like formation. Submucosa shows so-called angiomatous pools of blood and a focus of cellular infiltration.



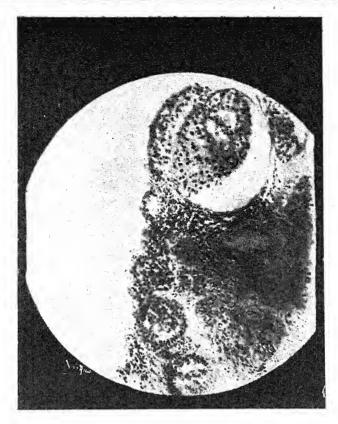


Fig. 1 imes 143 shows epithelial nodes in various stages of development and degeneration. The swollen node, about to be shed, shows a few peculiar nucleated bodies in the thinned central area.

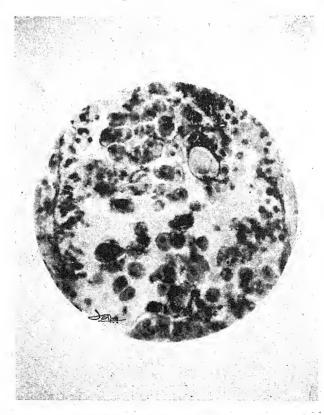


Fig.  $2 \times 216$  shows a colony of large-sized parasites and a double-contoured cyst, localised in an extremely attenuated enithelial node on the point of bursting.



Fig. 1 imes 335. Blood vessel shows a few parasites in the lumen and wall.

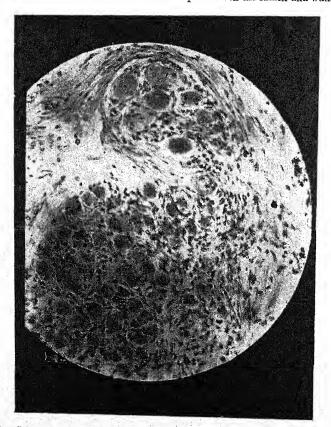


Fig.  $2 \times 150$ . Colony of quiescent parasites in broken down blood vessels. Note the subacute

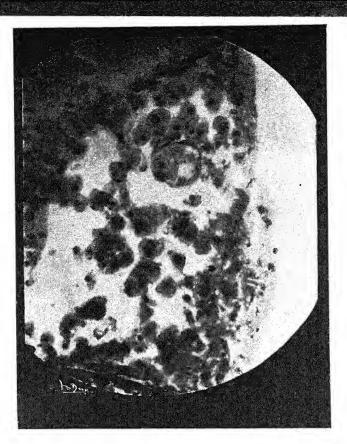


Fig. 1  $\times$  216. Colony of active parasites in the superficial lesion. Note the cyst, showing one definite, and two hazy nuclei, with a suggestive empty space.

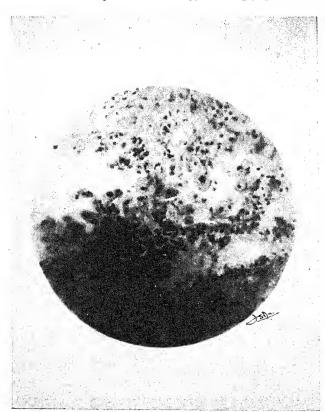


Fig. 2 × 129 Another colony. Note the leteral nuclear street on the lawer remaits on the ton right

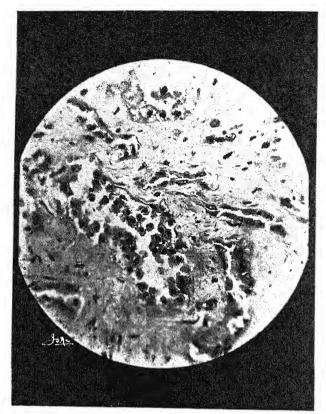


Fig. 1  $\times$  166. Section of the kidney dilated blood vessel shows parasites in the tumen. Note the difference in the appearance of the parasites, compared to the endothelial and other cells in the field.

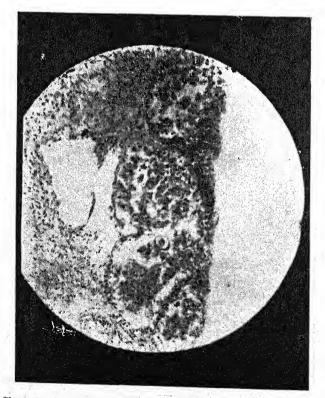


Fig. 2  $\times$  128. Shows four neighbouring colonies of rapidly multiplying parasites below the bladder surface. Note two cysts of varying shapes



Fig. 1  $\times$  335. High power photograph of a section stained with iron-hæmatoxylin, showing inclusion bodies inside the parasite.

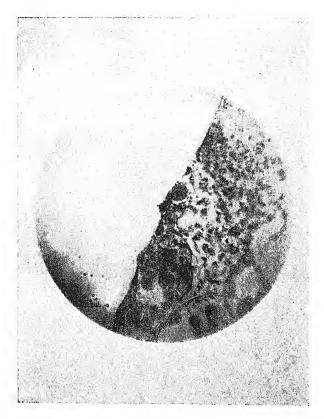


Fig.  $2 \times 244$ . High power photograph showing a colony of large-sized parasites in the superficial lesion in the bladder. Section was stained intensely by Gram's method. Note the typical character of

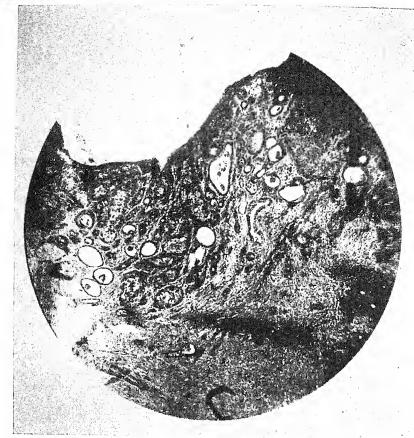


Fig. 1. Shows growths in the mucosa and submucosa resembling to an extent adeno-carcinoma.

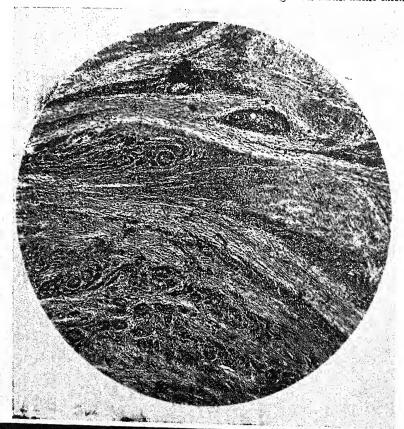




Fig. 1  $\times$  56. Low power photograph. Section of bladder shows two altered vessels. In one, the parasites still persist, while in the other they have been digested by body tissues.

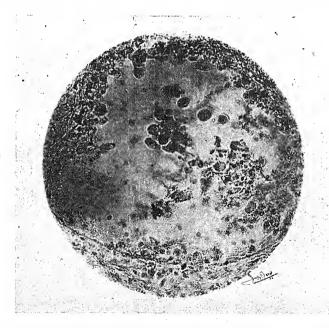


Fig.  $2\times244$ . A degenerated area in the duodenal lesion (Plate XIX, Fig. 2) showing a few parasites with the typical nuclei.



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# SOME OBSERVATIONS ON THE TRYPANOSOMES OF CATTLE IN SOUTH INDIA.

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### INTRODUCTION.

Only two kinds of trypanosomes have been met with in the blood of cattle in South India, viz., a large one resembling T. theileri Laveran, 1902, and a smaller one resembling T. evansi [ Steel 1885 ], and the same two trypanosomes are found in Northern India. Stirling [ 1920 ] appears to have found trypanosomes resembling T. congolense Broden, 1904 [ T. dimorphon Laveran and Mesnil, 1904 ], in a bullock that died in the Central Provinces under conditions suggestive of piroplasmosis, but the existence of this trypanosome in India has not been confirmed.

In the smaller trypanosome of cattle resembling *T. evansi*, which is also found in other animals such as the horse, dog and buffalo, no polymorphism seems to exist. Whether this smaller trypanosome as found in cattle is pathogenic to them and to

other domesticated animals, particularly to the horse, is a question which is often asked. Until recently it was commonly believed that such trypanosomes of cattle, in this part of India, were not very pathogenic to bovines and buffaloes, but with the increased facilities now available for routine microscopical examination of blood of cattle in the field, it has been possible to determine that definite outbreaks of trypanosomiasis do occur in bovines and buffaloes, causing mortality, which varies according to the severity of the outbreak. Edwards [1928] says that virulent outbreaks of surra occur among cattle and notably among buffaloes in which the mortality rate may vary from 20 to 80 per cent.

Other workers have shown that there exists some circumstantial evidence that, under natural conditions, these trypanosomes may be transmitted to horses, from bovines as reservoirs. But experimental evidence appears to be very meagre to prove the identity of these monomorphic trypanosomes of cattle and that bovines act as the reservoirs from which the disease is carried over from one season to the next, and to the horse, in which surra is fatal.

# EARLY EXPERIMENTAL WORK ON PATHOGENICITY OF CATTLE TRYPANOSOMES TO HORSES.

Lingard [ 1899 ], in his Report on Surra, mentions two horses which, on being inoculated with cattle trypanosomes, developed clinical symptoms of surra, with the appearance of the parasites in the peripheral blood. These two cases were briefly as follows:—

- (1) Australian mare, aged.—Inoculated subcutaneously, on 3rd October 1892, with 0.5 c.c. of blood swarming with trypanosomes taken from a cow. The latent period occupied 5 days and the temperature gradually rose during this time, the parasites appearing in the blood on the 6th day. Death occurred on the 14th day of the disease.
- (2) Horse.—Inoculated with 0.5 c. c. of blood swarming with trypanosomes taken from a cow. Result positive (but no details given).

Presumably on the basis of the two foregoing and perhaps other similar observations, Lingard [1906] was led to deduce the following conclusions: "Horses inoculated with blood in small quantities drawn from plains and hill bovines during the early stages of the disease, whether the blood be collected during a paroxysm or an intermission, contract the disease in virulent form, and death may take place when the infective blood is derived from a plains animal within the short period of 5 days, and when from hill bovines between a period of 19 or more days. When, however, inoculations are conducted with blood taken at a later stage of the disease,

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from a similar source, the course of the malady in equines is more prolonged, but equally fatal in its results."

Proof of the pathogenicity of cattle trypanosomes to equines is afforded by the results of two experiments carried out by Fraser and Symmonds [1909] in the Federated Malay States. The full account of these two cases, as given by the authors, is of sufficient interest to bear quotation:—

- (1) "In order to follow the course of the disease in a horse inoculated from a naturally infected bullock, an Australian mare, 9 years old, about 16 hands high and in excellent condition, was inoculated subcutaneously with 1 c.c. of blood from a naturally infected bullock. Seven days later the temperature of the horse rose to 103°F., and on the following morning trypanosomes were discovered. Thereafter the temperature was irregular, a remittent temperature of from two to five days duration alternated with an interval of one to two days, when the temperature was either intermittent or normal. During the last 12 days of the disease, the temperature remained constantly elevated. On six occasions parasites could not be found, their period of absence lasting from one to four days. Oedema of the legs was first noted on the 10th day and by the 13th day was marked; on the 18th day oedema under the abdomen was observed and later on it extended along the thorax. An urticarial eruption occurred from time to time. Loss of condition was pronounced by the 25th day and weakness of the hindquarters, which was first noticed on the 44th day, became very evident by the 50th day. On the 62nd day the mare fell down and did not rise again. Death occurred on the 66th day."
- (2) "An Australian gelding, 8 years old, in good condition, was inoculated subcutaneously with 0.1 c.c. of blood from a naturally infected bullock. The period of incubation was six days and the disease ran its course in 51 days. The observations as regards parasites and clinical signs were similar to those in the preceding case, save that oedema was never present. That this was not due to a different species of trypanosome was shown by the fact that another horse which was inoculated with the same strain of trypanosomes developed oedema of the legs, abdomen and thorax".

Fraser also conducted experiments to determine the identity of the trypanosomes encountered in other species of animals and, as a result of these experiments, came to the conclusion that the trypanosomes "met with in horses, cattle, and dogs are indistinguishable morphologically and in their pathological effects (and hence they all belong to the species *Trypanosoma evansi*)".

THE IDENTITY OF THE SMALLER TRYPANOSOME OF CATTLE IN SOUTH INDIA.

- (a) Morphology. The general morpological characters of this trypanosome agree with those described for T. evansi [ Steel 1885 ]. It is monomorphic with a well developed undulating membrane and a free flagellum. The measurements range from 18 to 33  $\mu$  in length and 1-5 to 20  $\mu$  in width. The average length is about 25  $\mu$ .
- (b) Biometry. The idea of the study of biometry was obtained from the work on Trypanosome Diseases of Domesticated Animals in Uganda by Bruce and his collaborators [1911] and also from Fig. 196 in "Protozoology" by Wenyon [1926]. In the latter two curves, one for T. evansi and the other for T. brucei, are given representing the percentage of trypanosomes in respect to length. It is presumed that those curves were plotted from smears obtained from animals in which relapses had occurred. Wenyon [1926] suggests that the notch, which these curves show, is an indication that in such infections two types of trypanosomes are present—not necessarily two species, but that the one species tends to produce in its development two main groups of organisms and it is of interest that curves drawn for T. evansi in white rats also show similar notches. Taliaferro [ 1930 ] says that in mice, where no crisis occurs, the infected trypanosomes do not come in contact with the lytic anti-bodies, hence they do not become biologically changed as they do after each trypanolytic crisis in such hosts as guinea pigs, dogs, etc. White rats behave exactly like mice in having no crisis when injected with T. evansi, yet a notch in the curve of lengths of trypanosomes is seen. Even the curves drawn for relapse strains of a known original, all maintained in different rats, also show a similar notch in each curve drawn. Curves drawn for a strain maintained in a guinea pig also show similar notches in the original as well as in those drawn during each relapse. Hence it would appear that the indication of such a notch, if it is of any significance, is that these trypanosomes have in them a latent possibility of variation which may manifest itself even without the action of a trypanolytic antibody. Ritz [1914] was able to produce twenty-two variants immunologically different from the original strain with which he worked and it seems that there are unknown factors which influence the virulence of the same type of trypanosome when it is obtained from different sources such as the bullock, dog, or horse, about which reference will be made later.

The biometric curves discussed in this paper were drawn from a large number of blood smears from different classes of naturally infected animals, received in this laboratory, and from the blood smears of experimental animals used in the present study. Since all these smears were made and stained under similar conditions, it is suggested that any error due to contraction of the parasite, etc., should be common to all the organisms drawn for measurement, and can be ignored. Fig. I shows

8 5 6 5 4 E 6

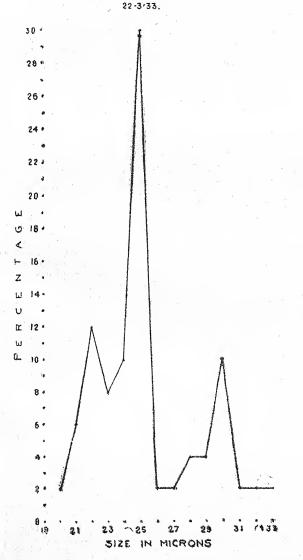
PERCENTAGE

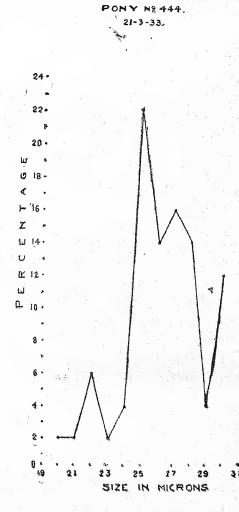
curves for trypanosomes in naturally infected ponies, dogs, cattle and buffaloes. The similarity in the rise of particular lengths is striking in all these curves. These curves also show that the average length of 25  $\mu$  is common to all and coincides with the average length of T. evansi. It was also found that the curves drawn for trypanosomes in experimentally infected ponies resembled those drawn for the trypanosomes from naturally infected ponies in respect of the average length and the range of variation in length. (Fig. II). In experimentally infected white

Fig. II.

Tryps: Bovine Strain
PONY Ng 444

Tryps: Bovine Strain





rats, however, the peaks of curves showed a predominance of trypanosomes measuring 30  $\mu$  irrespective of the fact of those trypanosomes being derived from a pony, a dog or a calf. (Fig. IV). It is not clear why the average length should come up to 30  $\mu$  in the white rat alone, while the same strain when injected into a dog, a pony, a calf or a guinea pig (Fig. III) gives an average length of 25  $\mu$ . It is suggested that this apparent increase in the variation of the average length of the trypanosome in the rat may be due to more uniform multiplication of trypanosomes in such an animal which offers no resistance whatever to rapid increase. The uniformity of the curves of the lengths of trypanosomes in white rats, irrespective of their source being from a pony, a dog or a calf is however significant. The similarities of the biometric curves suggest that those trypanosomes are identical and that, as the average length and the range of variation in their lengths coincide with T. evansi they are identical with it.

Fig. III. Tryps: Equine Relapse Strain.

Guineapig No. 508. 25-8-33.

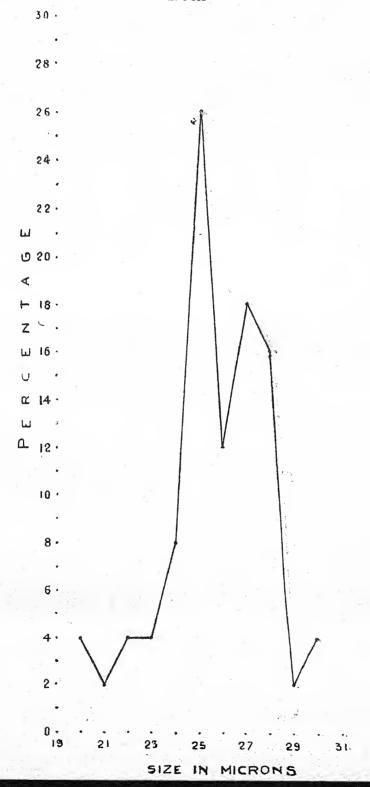
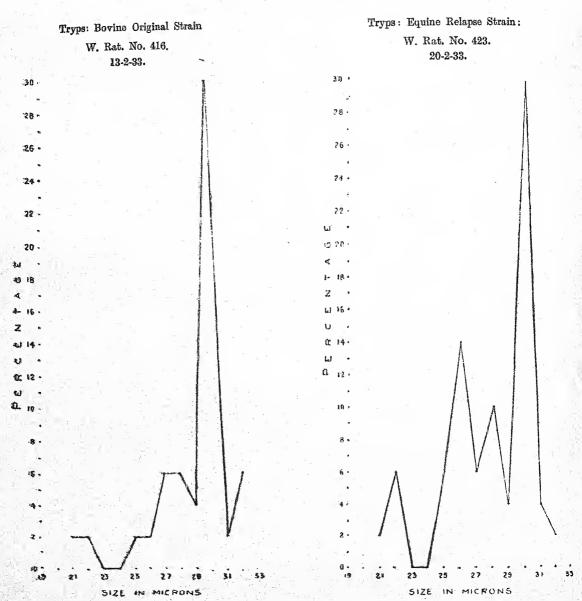


Fig. IV.



## PATHOGENICITY TO EXPERIMENTAL ANIMALS.

C135442119

(a) White rats. In the year 1929-30, in a long series of passages of the strains of these trypanosomes from cattle, dogs and ponies, it was found that in each

strain, when passed through a series of rats, the virulence was exalted to that animal. In each rat injected trypanosomes appeared uniformly on the 2nd or 3rd day and the rat died by the 4th or 6th day. All these strains appeared as if they became "fixed" in their virulence to the white rat. Similar results were obtained with the new strains of trypanosomes obtained during the year 1933-34. Natten Larier and Noyer [1931], while working with the trypanosome of the dromodery and the horse in Morocco, had the same experience with rats. Laveran and Mesnil [1907] observed that white rats when injected subcutaneously with T evansishowed them in their blood in 3 to  $3\frac{1}{2}$  days and died in  $5\frac{1}{2}$  to  $6\frac{1}{2}$  days and quote other workers who obtained similar results. All seem to agree that in white rats no relapses occur.

(b) Guinea pig. In this animal the course of the disease is subacute with relapses and they usually die in about 8 to 12 weeks after infection. These results are in conformity with those of other workers.

When a strain of trypanosomes, in passage through guinea pigs, was passed through a rabbit, it became more virulent to the guinea pig during the first or second sub-passage. Terry [1910] observed that a strain of Mauritian surra, when kept exclusively in guinea pigs, became attenuated for mice. The study of passages of the South Indian strain of *T. evansi* did not show any such attenuation to white rats.

Laveran [1908] has shown that the virulence of the Mauritian strain of surra when passaged through a series of guinea pigs may become very virulent to it. During the present study, it was remarkable that an equine strain, during its passage through guinea pigs, suddenly became very virulent to it and subsequently resumed its original character after three sub-passages. In the course of this period of exalted virulence, it was possible to infect two hyper-immunised rats, against the same strain, to which they succumbed. It is not understood how and why the virulence flared up to this height, though temporarily. The first of these three guinea pigs died within 24 hours after infection and the other two in 10 days after infection without showing any relapses. This is unusual in this animal, when injected with an ordinary strain.

(c) Ponies. These animals were used to study the pathogenicity of the bovine and canine strains on them. Five ponies were used. Each pony on purchase was kept under observation and its blood, in massive doses, was injected into two rats to make sure that it was free from trypanosomiasis. The ponies were kept in fly-proof stables.



The results of inoculation are tabulated below: -

### PROTOCOL I.

**************************************				
Pony No.	Source of strain	Incubation period	Duration of disease	Remarks
444	Bovine strain after 10th passage in white rats, ½ c. c. of blood subentaneously in neck.	6 days. (16th to 21st March 1933.)	30 days. Died on 15th April 1933.	Relapses two-one on 26th March 1933 and other on 29th March 1933.
419	Bovine strain from guinea pig, 415, \(\frac{1}{2}\) e. c. of blood subcutaneously in neck.		36 days. Died on 13th March 1933.	Relapses two—one on 28th February 1933 and other on 1st March 1933.
519	Bovine strain after passage through dog, 528, ½ c. c. of blood subcutaneously in neck.	4 days. (2nd to 5th	28 days.	
449	Canine strain from white rat, 452, after 10th passage. Le c of blood subcutaneously.		9 days. Died on 11th April 1933 with cerebral symp- toms.	No relapse.
296	Canine strain from dog.  i.e. e. of blood subcuta- neously.	5 days. (20th to 25th August 1930.)	30 days. Died on 22nd September 1930.	

It may be seen from the above protocol that the bovine or canine strain of trypanosomes is virulent to the horse and it seems that the canine strain is more virulent than the bovine. It also seems that a strain of trypanosomes after passage through a highly susceptible animal like the white rat becomes exalted in its virulence to the horse. Fraser and Symmonds [1909] also found that a pony injected with a canine strain of T, evansi died in about 8 days after showing trypanosomes in its blood.

(d) Cattle. Three calves were utilised in testing on them the virulence of an equine and a canine strain. The results are tabulated below:—

### PROTOCOL II.

Calf No.	Source of strain	Period of incubation	Duration of disease	Remarks		
418	Canine strain from a naturally infected dog, 1 c. c. of blood subcuta-	(3rd to 15th				
516	neously. Equine strain from white rat, 514, ½ cc. of blood subcutaneously.		22nd April 1933. Still alive .	afterwards. Trypanosomes seen from 7th to 13th September 1933. Negative ever		
284	Cauine strain from dog after passage. 1 c. c. of plood subcutaneously.	1933.) 8 days. (7th to 15th August 1930.)	Developed rin- derpest and died on 19th August 1930.			

In calf No. 418 very few trypanosomes were found on one day only in a wet preparation, yet its blood was found to be infective to white rats after the disappearance of the trypanosomes from its blood, but only when over 2 c.c. of blood was injected.

The blood of calf No. 516, though it was found negative under the microscope from 14th September 1933 onwards, was found infective to white rats for 60 days after the disappearance of the trypanosomes, but only when over 2 c.c. of blood was injected.

In the healthy calves used in these experiments, trypanosomes disappeared from their peripheral blood within a few days and very small doses of blood from these calves proved non-infective to such susceptible animals as white rats. This finding has an important bearing on the question of cattle acting as reservoirs in bridging the gulf between one surra season and the next. This question will be dealt with separately later.

Calf No. 516 is still alive at the time of writing (about 10 months after infection) and its blood is non-infective now to white rats even in massive doses. The blood of this animal is being tested periodically for adhesion phenomenon and it has completely lost that property, the loss being a gradual one.

(c) Dogs. Seven dogs were injected with equine, bovine, and canine strains, and the details are shown in the Protocol below. Four others, Nos. 275–276, 277 and 278 were infected for the study of adhesion phenomenon and two were treated with 'Bayer 205' to see if they could be reinfected with the surra trypanosome. Of these two, one (275) was reinfected successfully after sixty-two days and the other (277) died a few days after treatment.

# PROTOCOL III.

Dog No.	Source of strain	Period of incubation	Duration of disease before death	Remarks		
527	Equine strain from calf, 516.	5 days. (9th to 13th September 1933.)	28 days	Relapses two.		
528	Bovine strain from white rat.	6 days. (11th to 16th September 1933.)	54 days	Do.		
613	Bovine strain from bullock.	4 days. (14th to 18th February 1934.)	34 days	Do.		

Dog No.	Source of strain		Period of incubation	Duration of disease before death			Remarks	
288	Hound strain	•	5 days. (13th to 18th August 1930.)	9 days	•	•	No relapse.	*
289	Ditto .		Ditto .	23 days			Relapse one.	
301	Hound strain through pony.	passed	6 days. (28th August 1930 to 3rd September	19 days	•	•	No relapse.	
	ł		1930.)	1				
302	Ditto		3 days. (28th to 31st August 1930.)	24 days	•	٠	Relapse one.	

From the above it is concluded that strains of this trypanosome whether obtained from canines or equines, behave practically alike in the dog, but strains from bovines appear to be milder in virulence.

### CROSS IMMUNITY TESTS.

These tests were studied in white rats infected with different strains and cured with 'Bayer 205'. The dose of the drug per rat was fixed at 1 c.c. of a 0.5 per cent. solution given intraperitoneally. In all, 25 rats were used of which 19 remained refractory to reinfection with any of the strains used or their relapses. As many as 8 attempts were made, at intervals, to reinfect some of them, the last being seven months after treatment with 'Bayer 205'. Kasai and Akaza [1927] found that they could protect mice against surra for five months only by giving a prophylactic dose of 'Bayer 205' (0.005 gram). Thus after the lapse of seven months after treatment with 'Bayer 205' the experimental rats could not have had in their system any residue of the drug to prevent reinfection with the trypanosomes under investigation. The blood of all the nineteen rats referred to, when tested for adhesion phenomenon, proved positive. It is considered that these white rats had developed a true immunity against the trypanosomes under investigation. All the strains of trypanosomes under investigation failed to infect the immunised white rats, and it is concluded that these strains whether from dogs, horses or cattle belong to the same group, viz., T. evansi [Steel, 1885]. The experiments are summarised in Protocol IV.

In this connection some details of the history of the six white rats which showed a break-down in immunity will be of interest:

(a) White rat No. 525 was infected with a canine strain of trypanosomes on 16th February 1933, and treated with 'Bayer 205' on 21st February 1935, and treated with 'Bayer 205' on 21st February 1935, and treated with 'Bayer 205' on 21st February 1935, and treated with 'Bayer 205' on 21st February 1935, and treated with 'Bayer 205' on 21st February 1935, and treated with a canine strain of trypanosomes

- ary 1933. Eleven weeks after recovery, it was reinfected with a canine strain (passage) to which it succumbed.
- (b) White rat No. 437 was infected with a bovine strain of trypanosomes on 4th March 1933 and treated with 'Bayer 205' on 9th March 1933. Two months and five days after treatment it was injected with the same canine strain (passage) as in the previous rat (No. 525). This rat also died after taking the infection.
- (c) White rat No. 485 was infected with a bovine strain of trypanosomes on 25th May 1933 and treated with Bayer 205 on 29th May 1933. Attempts to reinfect this rat, between 10th July 1933 and 18th September 1933 either with bovine or equine strains of trypanosomes, failed, showing that it was immune to those strains. On 22nd September 1933, four days after the last attempt at infection, it was again injected with an equine strain of trypanosomes passed through a dog (No. 527) to which it succumbed.
- (d) White rat No. 510 was infected with an equine strain on 18th August 1933 and was treated with 'Bayer 205' on 22nd August 1933. Twenty-three days later it was injected with the same equine strain passed through a dog (No. 527) and the rat developed surra and died.
- (e) White rat No. 559 was infected with a bovine strain and was treated with 'Bayer 205' on 6th October 1933. Between 3rd October 1933 and 15th January 1934 it was injected twice with an equine strain of trypanosomes to which it resisted. On 16th January 1934 it was injected with an equine passage strain from guinea pig (No. 608) in which the trypanosomes had suddenly acquired an exalted virulence and succumbed.
- (f) White rat No. 537 was infected on 16th September 1933 with the n<sup>th</sup> relapse strain under passage in a guinea pig. It was treated on 20th September 1933 with 'Bayer 205'. It was reinfected successfully with the equine strain from guinea pig (No. 608) mentioned above. The interval between the time of treatment and reinfection was thus three months and 18 days and three months and 26 days respectively.

From the above it seems that the first four rats, did not possess any resistance to a canine strain of trypanosomes, or to a strain passed through a dog and it is inferred that a strain of *T. evansi* [Steel 1885], when passed through a dog, may become exalted in virulence and may produce the disease in white rats even though they possess a certain degree of immunity. That these white rats had some degree

of immunity is assumed from the fact that they resisted previous attempts to reinfect, while their blood when tested gave a strongly positive adhesion phenomenon even to the day of last reinfection.

The history of the last two white rats appears to show that a strain of trypanosomes, which has become exalted in virulence for some unknown reason, has the power of breaking down experimentally induced immunity and of producing infection when injected, into a susceptible animal.

The above findings also seem to show that in some animals acquired immunity may be overcome by certain strains of the same trypanosome depending upon the degree of virulence of the strain used and that 'Bayer 205' may not protect white rats beyond three weeks.

# ADHESION PHENOMENON.

The methods adopted in studying this phenomenon were as described by Taliaferro [1930], Davis and Brown [1927] and Duke and Wallace [1930] and a large number of tests were made with blood from experimental animals. Wet preparations were examined after 15 to 20 minutes. Three things were noticed in these preparations, viz., (1) Agglomeration of trypanosomes, (2) adhesion of platelets to trypanosomes, especially to their flagellae, and (3) adhesion of red blood cells in addition to platelets completely enveloping the trypanosomes, particularly in dogs' blood. Agglomeration of trypanosomes was a constant feature even when relapse strains were tested with their original passage strains in white rats or other animals. From the uniform success of the very large number of tests in obtaining positive adhesion and agglomeration, with different strains, it is concluded that these strains are identical. It appears, therefore, that the strains of trypanosomes found in Southern India are identical, and further that the trypanosome in question is identical with T. evansi.

It is of interest that only in a few instances was slight deviation seen in the degree of the adhesion phenomenon between the original strain and its relapses.

# RESERVOIRS OF SURRA.

By a 'reservoir' of surra, in the strictest sense of the term, it is meant that the disease is latent, so that the causal agent is carried over from one surra season to another in the system of an animal in apparently good health. Such an animal may not show trypanosomes in its blood, under the microscope, but it should be possible to demonstrate their presence by animal inoculation, during the non-surra season.

It is admitted, on all hands, that the surra season in India is between June and October, but sporadic cases have been known to occur during the so-called non-surra season.

In the camel, we have an accredited 'reservoir' of surra, because in that animal surra runs a chronic course extending over a period of three years, punctuated with frequent paroxysms, when T. evansi is demonstrable under the microscope. Hence, wherever camels are found, it is easy to conceive, that diseased camels act as foci of outbreaks of surra in horses or other animals, provided the agent believed to mechanically transmit those trypanosomes are present at the time. From the available literature on trypanosomiasis in India, it is found that any work done on this disease, including the question of fixing the probable reservoirs, has been done only in Northern India where the well-known reservoir—the camel—is present. In the Madras Presidency there are no camels and the question of cattle and buffaloes being the reservoirs has to be examined.

No work appears to have been done to find out if the wild animals which abound in India serve to bridge the gulf from one surra season to another and we have no knowledge of a real vector like the tse-tse fly in South Africa. Hence with a certain amount of reserve we draw our own conclusions as to the possible reservoir-hosts of surra.

Workers in Northern India have attempted to show that in addition to the camel, cattle and buffaloes are likely to be carriers.

Montgomery [1908] said that the virus of surra is found wherever the camel of Northern India abounds and there is a possibility of its existence wherever cattle and buffaloes exist, and until the contrary be proved all these may be considered as the possible hosts of the trypanosome. He further said that the conditions under which bovines work render them less dangerous than camels as originators of the disease, and as a class not so important as store-houses.

It may be pointed out here that in countries where camels — the admitted reservoirs — exist, the argument that such refractory animals as cattle also act as reservoirs appears to be of doubtful value, because the latter may have been infected from the camel and the infection may have caused death, or spontaneous recovery may have occurred within a few months. It is undoubtedly true that infected cattle showing trypanosomes in their blood, may, like all other animals, act as temporary foci of infection, but this does not prove that they really carry trypanosomes in their tissues from one surra season to another, to be the real reservoir hosts.

In the months of January and February 1934, it was possible to test, in the Madras Veterinary College Laboratory, the blood of 16 emaciated cart-bullocks

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brought to the College Hospital for treatment and of one calf experimentally inoculated with surra in September 1933. Many of these bullocks came from places over 20 or 30 miles away and some of them came from near Saidapet, where surra is known to break out periodically. January and February were the months chosen for these tests, because they are about the middle of the so-called 'non-surra' season. The blood of each was examined under the microscope and each sample of blood was tested for adhesion phenomenon before injection into white rats, each of which was injected with ½ c.c. of blood intra-peritoneally. The results are noted in the following table:—

PROTOGOL V.

Serial No.	No. of case sheet	Result of microscopical examination	roscopical adhesion animal		Remarks	
					) necessary and the second sec	
1	1134	Negative .	Negative	Negative	• •	
2	1240	***	Positive	23	• • • • • • • • • • • • • • • • • • • •	
3	1101	19	Doubtful	,,		
4	1266	"	Positive	,,		
5	1241	*	Doubtful	33		
6	1146		Positive	,,		
7	1224	,,	Negative	,,	****	
8	1141	94 ·	Positive	25	*	
9	1109	55 .	Negative	2)		
10	·	73	Doubtful			
11	1153	, , ,		77		
12	996	73	Negative	<b>33</b>		
13	983	59		»		
14	1225	59		72		
15	765	<b>,</b>		40		
16	895			* \( \lambda_1 \)		
17	Expl. calf 516		Positive	"	m 10 11	
			-030170	<b>,,</b>	This calf is still under observa- tion and is negative to tests.	

It may be seen that none of these harboured trypanosomes though five showed a positive adhesion phenomenon and the history of experimental calf No. 516, which had been kept in a fly proof stable, is of interest. This calf was injected on 4th September 1933 with an equine strain of trypanosomes, which appeared in its blood in large numbers from 7th September 1933 to 13th September 1933. After this date its blood was negative under the microscope for trypanosomes but remained infective to white rats from 13th September 1933 to 13th November 1933 whether given subcutaneously or intraperitoneally. This animal is still negative at the time of writing (11 months from the date of primary inoculation). The blood when tested for adhesion phenomenon in the early periods was strongly positive; but its strength has progressively diminished and is now The result of these observations, when compared with the positive adhesion tests of the other bullocks, may be interpreted to mean that those animals had had trypanosomiasis during the so-called surra season, a few months before the tests and had recovered. The failure to infect white rats indicate that none of these animals had latent trypanosomiasis and were not reservoirs of surra, but before drawing conclusions it will be of interest to discuss the observations of workers who hold that cattle and buffaloes are likely to act as reservoirs of surra between one and the next surra season.

Lingard [1899 and 1907] observed that when cattle infected with surra develop the disease, many recover spontaneously and a few die showing paroxysms just as in the horse, while the blood of those that recover becomes negative for trypansomes after the lapse of a few months to microscopical examination and to animal inoculation and remains so.

Pease [1906] injected 3 buffaloes with camel surra. Two died in the course of one and three months respectively and the 3rd was kept under observation for 786 days, during which period he found its blood to be infective to dogs from time to time. There is however one defect in this experiment in that the buffalo was apparently not protected from fresh infection.

Schien [1908] says that an experimentally infected buffalo remained infective to laboratory animals for 5 months only. Some time later he infected the same buffalo successfully with surra and it remained infective, to animal inoculation, only for 4½ months from the time of second injection. This finding of Schien contradicts what Pease had observed in India.

Cross and Patel [1922] injected camel surra into buffaloes and found that they neither lost condition nor did trypanosomes appear in their blood; their blood was found infective to animal inoculation only for 163 to 172 days after the primary infection. The observations of these workers are in agreement with those of Schien regarding the duration of the infective period in a buffalo.

Edwards [1928] encountered outbreaks of surra among buffaloes maintained for serum production at Muktesar and by the systematic examination of the blood of infected animals, he found that in these trypanosomes persisted for some months, relapsing in relatively small numbers in the blood stream at relatively prolonged intervals. Many of the naturally infected stock, therefore, appeared to be on the road to spontaneous recovery, which process in these animals is characterised by the recurrence of trypanosomes being progressively lessened and spaced by longer intervals. It is true that during such a period in the progress of recovery from trypanosomiasis in bovines or buffaloes a concomittant attack of rinderpest is capable of accentuating a relapse of the trypanosomes.

From these observations of Edwards it is clear that the trypanosomes may be made to relapse during the early phases of spontaneous recovery in cattle, but in the light of the observations of Lingard, Schien, Cross and Patel and our own, it seems that it is not possible to cause these trypanosomes to relapse in the later phases of recovery, thus resuscitation of trypanosomes in cattle seems possible only in the earlier phases of spontaneous recovery, unlike what happens in piroplasmosis, in which disease the majority of the animals are known to remain in a state of premunition, perhaps for life.

Lingard [1906], discussing the periodical outbreaks of surra in ponies in Bhugiaghat, one of the stages on the road from Kathgodam to Nainital brewery, says, "In some cases the disease no doubt may be first disseminated through flies carrying bovine or buffalo blood to the equines on the road; but this will not account for the first case of surra always appearing at one particular stable (Bhugiaghat) for the horses ran up 8 miles and continuously met bullock carts and military bullock trains one way or the other." He also remarks that when the old stable at Bhugiaghat was abandoned in favour of a new one, half a mile down the road, surra ceased to break out until the old stable was again occupied some time later.

Holmes [1908] writing on the outbreak of surra in Bhugiaghat said that surra broke out each time at this halting place alone, and not at the others, although near each halting place there existed a camping ground for bullock carts, etc. In the Bhugiaghat stable the first case of surra appeared when tabanus flies were becoming scarce.

Leese [1908], who was deputed to investigate the outbreak at Bhugiaghat, said that he injected the blood of four apparently healthy bovines into experimental animals and found one among them infective to experimental animals. He, therefore, opined that the probable reservoirs of surra were cattle.

The observations of these three workers suggest that though cattle and biting flies, believed to be the necessary factors to initiate an outbreak of surra in horses,

were present in every stable on the tonga line on the road between Kathgodam and Nainital, yet surra appeared year after year at the Bhugiaghat stable only. Another interesting feature was the subsidence of surra when the ponies were removed to a new stable at Bhugiaghat not far away from the old one, though the common factors necessary were present there too. Thus if cattle were the reservoirs in that area, surra should have appeared in other stables as well, and it is suggested that since cattle are liable to infection of surra, the finding of one infective bullock by Leese in the locality, during an outbreak, does not form a logical basis to conclude that cattle were the reservoirs from which originated the outbreak among the horses at Bhugiaghat, and at that place alone.

In trying to settle the question whether biting flies were chiefly responsible for the spread of surra, Leese [1909] chose Mohand, a locality well known as a surra zone where plenty of ponies died of surra, when a tonga service existed there. The condition of Mohand in connection with surra is best described in his own words.

"The traffic in the rainy season has been insufficient for years because of the construction of a railway to Dehra Dun; the tonga service has been abandoned now for about eight years, and the bullock cart traffic in the surra season is very small indeed. The only equine traffic is a drove or two daily of pack animals. Only the older inhabitants of Mohand know anything about surra, and although about ten ponies are kept by the villagers and are constantly in contact with the milch cows and buffaloes of the village, no losses now occur in the rains. This is interesting that owing to the scarcity of "reservoirs of infection" (i.e., cattle and buffaloes working through Mohand) the danger to local animals has been largely removed."

"Camels always leave this road from about the 15th May to October; none are kept in the neighbourhood between these dates."

"The country consists of first class jungle full of game, but the latter evidently do not act as reservoirs of infection to domesticated animals."

"I had a temporary stable put up a quarter of a mile from the village,.....
The disease did not occur in the village and the outbreak was confined to our stable."

Mohand village in Mohand Pass evidently has always been used as a halting place to bullock cart and pack-ponies traffic, and the conditions as regards the prevalence of biting flies and the existence of domesticated animals of the village have been constant factors. Surra broke out constantly among the ponies in the tonga service till the latter was terminated some years previously and from what Leese says it would appear that surra did not exist in the village at the time of his visit. If cattle and buffaloes were the reservoirs, then there should have been enough of them to initiate the outbreaks of surra among ponies of the tonga service

and also to infect other healthy cattle at that time in the presence of biting flies there. In other words, there should have been a few bovines there as surra reservoirs, and these few should have naturally acted as so many foci, in the presence of the biting flies, from which the next generation of cattle or the new arrivals could be infected to become reservoirs of surra in their turn from one surra season to another. But it would appear from the observations of Leese that there were no reservoirs of surra at the time of his visit and no losses occurred among the village ponies. Even if cattle and buffaloes working through the village were limited, yet at least one among these might have been expected to have become a reservoir of surra, and one is as good as many, in the spread of the disease when so many biting flies prevailed there during the surra season. This scarcity of reservoirs, observed by Leese, casts a doubt on the theory of cattle being the reservoirs of surra from one surra season to another, since Mohand appears to have been an ideal place for the formation of such reservoirs in working or milch cattle.

Leese [1909] admits that in cattle the trypanosomes of surra are scanty in their blood or apparently absent from it, thus reducing the chances of mechanical transmission practically to nothing and this has been proved experimentally by Cross and Patel [1922] who found that if the number of trypanosomes in the blood of an animal was less than five per field mechanical transmission through the agency of biting flies was not possible.

Leese created in Mohand a limited outbreak of surra in his experimental animals which were kept only two furlongs away from the village from June to the end of September, and he admitted that some of the village cattle strayed near the camp in spite of which the village ponies remained healthy though these mixed freely with the cattle. This he thought was due to the cattle remaining healthy,—an unlikely thing in the vicinity of a focus of surra and during the full swing of the fly season.

Fraser and Simmonds [1909] working in the Federated Malay States, an endemic area of surra, found that the blood of cattle whose temperature remained normal after an attack of surra, was non-infective to other experimental animals. They also found that surra in bovine did not confer immunity after recovery. From this finding the present authors deduce that recovered bovines are not in a state of "Premunition," a condition which should be present if cattle and buffaloes do act as reservoirs of surra.

In the fly belts of Africa, cattle appear to be in a state of premunition, as regards T. congolense and the like, because a cyclical development of these trypanosomes is known to occur in the tse-tse flies, the bites of which help to induce such a condition in cattle making them carriers of the disease. The conditions in India

are however not parellel and do not help us to derive at a conclusion on the point at issue, as regards *T. evansi* and its reservoir, particularly in view of our present limited knowledge of the "mechanical" vector concerned.

Sowerby [1911] conducted two series of experiments in Bombay to find out latent trypanosomiasis in buffaloes. He injected into guinea pigs one c.c. of blood from apparently healthy buffaloes slaughtered in Bandra. The first series of experiments were conducted in the 3rd week of April 1908 when he injected the blood of five buffaloes into guinea pigs, one of which developed trypanosomiasis in the middle of June, almost two months after the primary inoculation which appears to be an unusually long incubation period. In the second series, conducted from 11th May to the 16th July 1911, he injected the blood of 44 buffaloes into as many guinea pigs among which three developed trypanosomiasis with an incubation period ranging from 3 to 4 weeks. These experiments no doubt proved the possibility of buffaloes acting as reservoirs of surra, but it would appear that Sowerby had not satisfied himself that the buffaloes whose blood proved infective were not actually suffering from the disease at the time. In this connection, it is of interest to quote Lingard [1906] who observed that the first case of equine surra appeared in Bombay a few weeks after the onset of the monsoon (June and July) although sporadic cases occasionally occurred prior to that date in some portions of the province and it is suggested that Sowerby's buffaloes may have been in the early phases of spontaneous recovery after a natural attack of surra and may not have been actual reservoirs.

Gaiger in April 1911, conducted a few experiments to find out if cattle in the Pubjab acted as reservoirs of surra. He selected eight emaciated cattle from which he drew blood and injected it into rabbits. One among these developed surra after an incubation period of 9 days. This experiment suggests the possibility of latent trypanosomiasis in cattle, but it appears that Gaiger did not satisfy himself that that positive bovine was not in contact with surra camels which are common in that Province.

From the above discussion it appears that, though some workers have shown the possibility of bovines and buffaloes having latent trypanosomiasis, there is no evidence to prove that their condition remains unchanged from one surra season to another. In countries where camels abound, cattle may get infected from them and this fact is liable to vitiate the results of experiments.

It is well known that cattle are refractory to surra, comparatively speaking, and that a large majority of them recover spontaneously but in these animals trypanosomes may be found for a few months, after which they appear to become negative to animal inoculation. Hence in the light of the experimental evidence of

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the workers quoted above and of this laboratory, a doubt arises if cattle really act as agents in carrying trypanosomes of surra in their tissues from one surra season to the next.

Some workers claim to have discovered in bovines and buffaloes the so-called reservoirs of surra by infecting laboratory animals with a syringeful of blood which happened to be negative under the microscope at the time of testing. But this cannot be taken as proved for the reasons already stated. In nature a biting fly will carry on its proboscis, only a minute fraction of a drop of infective blood, and in that quantity a comparatively large number of trypanosomes must, as proved by Cross and Patel [1922], be present for successful infection. In the blood of cattle that have recovered there are very few trypanosomes, or none at all, and it seems doubtful if they could carry the disease over from one surra season to another. The question of the number of flies which feed on a bullock with latent trypanosomiasis and immediately feeding on a healthy animal has also to be considered. This may be dealt with by quoting Gaiger [1911] who drew a few drops of blood of a camel between paroxysms and injected into horses with negative results.

The present authors therefore agree with Montgomery [ 1908 ] that bovines are less dangerous than camels as foci of infection and much less important as storehouses and wish to add that more experimental evidence is needed to class them as reservoirs of surra to bridge over the period from one surra season to another. There is no evidence to show that game animals in India are not reservoirs of surra and work in this direction appears to be required.

#### MECHANICAL TRANSMISSION BY TABANUS.

In Northern India, and in some other parts of the world, a few workers have shown that it is possible to mechanically transmit surra from one animal to another through the agency of certain blood sucking flies. From the literature available on this subject, it appears that there is hardly any statement made by one worker, regarding the power in mechanical transmission of any one fly, which is not contradicted by another with experimental evidence. In spite of so much conflicting experimental evidence, the power of the Tabanidae to act as mechanical carriers or transmitters is considered to be well established by many workers, particularly by Leese, who spent some time in India on this problem. In his remarks on the outbreak of surra in Bhugiaghat stable in 1907, Leese [1908] said that the disease was discovered at a time when tabanus was certainly absent. He found that cessation of the outbreak corresponded with the diminution in the number of stomoxys and also with the diminution in the number of blood marks caused by some 'undetermined' fly, which evidently bit all classes of animals and made no particular

choice of animals to feed on. Holmes [1908] says that there are grounds for concluding that stomoxys could not have been the sole agent in the transmission of the disease, because stomoxys attacked the healthy horses working in contact with diseased ones during the day and nothing happened.

Leese [1909] said that the general distribution of stomoxys is an argument against its being the first cause of an outbreak in horses, because surra in horses is not so generally distributed. In his opinion, stomoxys is unlikely to act that way and as cattle usually have the trypanosomes so scanty in the blood or apparently absent from it, the chances of this fly transmitting surra from an ox to a horse are reduced to zero. The same author [Leese 1912] says tabanus also has a very wide distribution though not to the same extent as stomoxys. Hence what has been said about stomoxys applies equally well to tabanus flies in relation to their distribution and outbreaks of surra. Leese himself has said that surra does not appear in horses in every fly zone. It would appear therefore that the abundance and distribution of tabanus flies have no exact bearing on these outbreaks.

Leese has observed the spread of surra in camels or in horses in the absence of tabanus but in the presence of other biting flies, yet he appears to hold that tabanus flies are the most dangerous though adequate arguments are wanting to prove this.

Pease [1884] in his report on surra stated that in the Bombay Tramway outbreak, the animals attacked with surra were not in close proximity to each other, but were dotted over the large area which the stables covered and were surrounded by healthy animals, which remained free and he concluded that animals suffering from the disease were not a source of danger to healthy animals.

Holmes [1906] said that in Muktesar Laboratory during the surra season, numerous green coloured tabanidae infested the stables and were seen to feed on infected ponies for a time till disturbed and immediately fed on healthy ones, kept in the same loose box. He observed this for three years running and no spontaneous case of surra occurred. He further said that the theory of such biting flies mechanically transmitting the disease in cases of natural infection looked less probable and less worthy of consideration from a practical standpoint.

Allen [1930] has reported on the outbreak of equine surra in Mona Remount Depot in December when there were no tabanus flies present there. He failed to secure even a single specimen of tabanus during the course of the outbreak although a careful search was made for them daily. He quotes a mass of literature which indicates that the observations made on the common species of tabanidae definitely show that the seasonal abundance of these flies varies within wide limits according to the species, and the localities in which they appear. Hence it seems that the

generalisation that tabanidae are the main factor in the transmission of surra and more especially the cause of the first case in an outbreak is inadmissible. There have been more unsuccessful experiments in transmission of surra with biting flies in a laboratory than successful ones, but none excepting Cross and Patel [1922], seems to have explained the cause of the failures. At any rate successful transmission does not explain how the first case occurred in an outbreak especially in South India where the admitted reservoir, the camel, does not exist. The question therefore arises why tabanus flies alone are to be looked upon as the most important ones concerned and not other biting flies. It has been proved by various workers that no cyclical development of *T. evansi* takes place in the tabanus, stomoxys, haematopota, etc., and it has been shown that even in the absence of these flies outbreaks of surra are encountered.

Patton [1909] described crithidia tabani in the gut contents of some of the tabanus flies caught by him. He doubted the existence of any connection between these crithidia with any hæmoflagellates and opined that new flies got infected with the post-flagellate stages passed in faeces on places where they rested. While working in Mesopotamia he found similar crithidia in hundred per cent. of Tabanus glaber during an outbreak of surra in camels in one of the camps. He observed that these gad flies fed on the dromoderies and in so doing they ejected a dark fluid from their alimentary tracts, sometimes pure blood, on to the skin and hair of the animals. This fluid he examined and found to contain crithidia and their post-flagellate stages. The dromoderies were noticed to drive away the gad flies by licking the areas where they were bitten and at each of such acts the material infected their lips. It therefore occurred to him that the trypanosomes found in the dromaderies may be but a stage of the crithidia parasitic in the flies and that when these crithidia, etc., came in contact with the mucous membrane of the mouth, they penetrated it and entered the blood stream, to assume the trypanosome form. With this in view he applied to the mucous membrane of the mouth of two young camels the gut contents of the tabanus, but he lost touch with these experimental animals owing to change in camp and the results are not known.

In 1931 one of us (M. A. Rao) had an opportunity of visiting Kaveripakam, Ranipet, etc., where outbreaks of surra among ponies had been observed periodically for some years. A few cases of surra appeared in ponies in February 1931, and it was possible to visit the area in the month of May, when it was found that there were a very large number of tabanus flies present. Kaveripakam in particular was full of these flies and within sixty minutes over 65 T. striatus, 5 T. ditaeneatus, 2 T. virgo, a few hyperosia and stomoxys were collected. Most of the T. striatus were caught on the trunks of trees near which ponies and cattle were tethered. The people here have no stables for their ponies and these are kept in the open under the

shade of trees. Some of the tabanus and the other flies were caught on the bodies of animals in the act of feeding. Of these gad flies thirty-five were dissected and ten of them possessed crithidia and their post-flagellate form in their gut contents. An emulsion of these was injected into a white rat subcutaneously and brought to the College Laboratory, Madras, via Ranipet where twelve more T. striatus were caught in the cattle sheds of the market. The other two species of Tabanus, lyperosia and stomovys were found free from any flagellates in their intestinal contents. The remaining tabanus flies were divided into two batches and each batch was put in a wide mouthed bottle and applied to a dog and a calf for six days consecutively. The majority of these did not feed and the mortality rate being high, they all died out in six days. Those that fed on the dog and calf passed faeces or even blood through the alimentary canal on the bodies of those two animals which they licked after the withdrawal of the flies. The rat and these two animals were under observation for over two months and did not develop surra.

Kaveripakam was again visited in July of the same year and though the flies were scarce at the time, over 88 T. striatus were caught while feeding on bullocks and ponies. The blood of these animals (8 pairs of bullocks and six ponies) was found to be negative for trypanosomes. These flies were brought to the College Laboratory in a cage covered with netting. 25 flies were dissected and only four were found infected with crithidia. Two white rats were injected with one c. c. of the emulsion which contained an average of three crithidia per field under the microscope and the result was negative. A few flies were allowed to feed on a white rat which was kept under observation for 23 days with negative results. One bull calf was injected subcutaneously with 2 c. c. of the emulsion of the gut contents containing on an average of six crithidia per field under the microscope and another calf was injected intravenously with the same quantity of the emulsion. These calves were under observation for a month and they did not develop surra. The blood of these calves was examined under the microscope periodically for T. evansi and at the same time cultures of the blood were made to find if T. theileri could be obtained but results were negative to develop it.

Our observations on the crithidia parasitic in the gut of tabanus show that they are usually present only in the alimentary tracts of those gad flies that have fed on blood. Patton [1909] has observed that a large percentage of female tabanus which he caught and dissected had not fed on blood and yet their eggs were mature and that they had some other food was indicated by the presence of a yellowish fluid containing yeast-like cells, vegetables fibres, bacteria and gregarine cysts. In his observations on flies, which did not feed on blood, he was not able to find the parasitic crithidia. This supports our finding that blood is essential for the development of these crithidia from whatever source they may have been



ingested by the flies. The sources of infection in flies may be the post-flagellate stage passed in facces of flies as suggested by Patton and Cragg [1913] or it may be from the latex of certain plants as suggested by Davidson quoted by Warren [1932] with regard to the tsetse fly, or it may be from the blood of an animal containing hemoflagellates. The necessity of blood for their development in the alimentary tract of Tabanus appears to suggest that there is some connection between these crithidia and a hæmoflagellate. Patton and Cragg [1913] said that there are two broods of tabanus flies in Guindy (Madras). If this were so, a certain number of months would elapse between the 1st and the 2nd brood, during which period no flies would be met with. Hence, even if the 1st brood had contaminated the blades of grass overhanging water or other places with the post-flagellate stages of the crithidia in their facces, these could not have remained viable long enough under the conditions of heat, etc., in South India, nor could the blades of grass remain unaltered during the period. It therefore seems unlikely that the source of infection is from the faeces of the previous brood as suggested by Patton and Cragg. It is also unlikely that these flies pick up the infection from the latex of certain plants which become evident only after a feed on blood later. This remains to be proved. In any case, such a supposition seems improbable because no leptomonas or phytomonas have yet been seen in the female flies which had not fed on blood or in male flies which are supposed to feed on juices of plants and flowers. The negative results obtained by us after injecting the crithidia and their post-flagellate forms into experimental animals, suggest that these may not have any connection with surra.

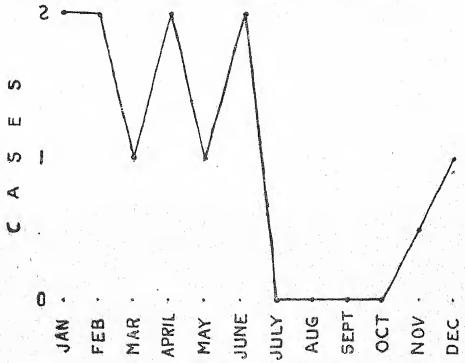
Old [1909] suggested that the ova or larvae of the tabanidae, eaten with grass might give rise to trypanosomiasis through a stage latent in them. This suggestion was tested during the present investigation by feeding a pony and a few white rats with ova of tabanus collected from grass on the banks of the Adyar river near Saidapet and Guindy (Madras) where surra breaks out periodically among ponies. The results of these experiments were negative. There is a belief among people here that when ponies are fed with grass cut from the banks of streams they contract surra and die. This looks doubtful in the light of our observations and further verification. The experimental evidence detailed above confirms that no cyclical development of T. evansi takes place in Tabanus. Leese [1908] observed in Bikaner that when Lyperosia in plenty were biting camels, they rubbed against each other and the blood drawn out at the punctures, in an infected animal, may have infected similar punctures in the healthy ones. The spread of the disease in Mesopotamia seen by Patton [1920], may have been due to such a thing which may have escaped his notice at the time and not by the ingestion of the faeces of the flies containing crithidia, etc., as suggested by him.

A graph of surra cases detected in ponies brought for treatment at the Ranipet Veterinary Hospital is appended (Fig. V.). A reference to this shows

Fig. V.

Positive cases of surra in ponies recorded at Ranipet Veterinary Hospital, 1928 to 1932.

Note the absence of cases in the so-called surra season.



that no cases of surra occured during the so-called surra season and this coincides with the observations of Allen [1930] at the Mona Remount Depot. Hence it would appear that tabanus flies need not be important vectors in natural outbreaks of surra in horses or other animals. Most workers in discussing the genesis of outbreaks of surra in animals appear to be satisfied in assuming the hypothesis that the trypanosomes were derived from a bovine, buffalo or a camel by Tabanus flies, but the experimental evidence, if any, put forward by them is not convincing. It has been shown in the preceding pages that there

is not much evidence to definitely prove that bovines and buffaloes are the reservoirs of surra, at any rate in the Madras Presidency, where camels are absent though there is the possibility of those animals being foci of infection in the early phases of the disease in them.

OBSERVATIONS ON THE OCCURRENCE OF TRYPANOSOMIASIS IN RINDERPEST VIRUS PRODUCING CALVES.

A statement is appended noting the number of cases showing trypanosomes in the virus producing calves and also the percentage of infection of surra and piroplasmosis in them. The virus producing calves are housed well and no tabanus flies have been seen biting them though stomoxys flies are almost always present. The statement shows that all the attacks in each year were during the so-called surra season-July to October-and some cases have been noted from November to the end of February. It is possible that most of these calves purchased during the surra season were incubating trypanosomiasis, or were in the early phases of spontaneous recovery at the time of purchase. In these therefore, the trypanosomes relapsed when they were infected with rinderpest. The fact that no cases of trypanosomiasis occured between March and June is significant in showing that none of the calves used then were in a state of premunition. This period apparently should be the gulf between one surra season and the next, and therefore among the calves no "reservoirs" existed then. It is known that surra sometimes may exceptionally break out late in the cold season (December) and when this happens, some of the animals in the early phases of recovery may show up trypanosomes in January, February or even later when infected with rinderpest. Such exceptions should however not be taken as evidence to prove the converse and there is evidence that cattle are not in state of premunition after recovery. Hence it is doubtful if they can be made to relapse excepting during the early phases of spontaneous recovery and it seems doubtful from the above observations, if bovines can act as carriers of surra, in the strictest sense of the term from one season to the next.

PROTOCOL VI.

Statement of occurrence of trypanosomiasis among virus producing calves.

	1928-29.	1929-30.	1930-31.	1931-32.	1932-33.	Romarks.
March						
May		•		••		

Photogramme	1928-29.	1929-30,	1930-31.	1931-32.	1932-33.	Remarks.
July	• •		2		• •	
August	••	-15	••			
September		7		1		X-1
October	·	3	2		1	
November	••				2	_
December	••	1		* -	5	
January	1	••	* ··	••	••	1) 1
February	1	1		•••	$2^{^{\times}}$	0
Percentage of Trypanosome infection	0.45	6.0	0*7	o•3	2·1	Total 9·55
Percentage of Piroplasma infection	20.0	11.0	<b>15</b> •0	17.0	* 17•5	,, 80.5
Total number of calves used	218	478	454	367	427	,, 19 <b>4</b> 4

THE LARGE TRYPANOSOME IN THE BLOOD OF BOVINES AND BUFFALOES (T. theileri LAVERAN 1902).

In Northern India Durrant and Holmes [1904] appear to be the workers who first described the large trypanosome in the blood of a bull and they observed that that bull had diarrhoea, intermittent fever, loss of appetite, staring coat and anæmia. Six days after the development of the symptoms, they found the animal dead.

Lingard [1907] was the next who wrote on this giant trypanosome in the blood of cattle in Northern India and gave it different names, viz., T. himalayanum, T. indicum, T. mukteseri, etc., which are now taken as synonyms to T. theileri Laveran, [1902]. Lingard found them mostly in hill cattle injected with rinderpest blood and not in the blood of plains cattle or in untreated hill cattle. He observed urticarial eruptions on the bodies of some of those that showed these trypanosomes.

Valladares [1908] found this trypanosome in the blood of a Nellore-bred bull in the Madras Presidency and recorded the symptoms he observed in this case. It had fever, urticarial eruption on body, was off its feed, dull, listless and exhibited a tendency to lie down in preference to being on its feet. The spine was arched, and the animal was severely constipated. It died within a couple of days after it came under observation. Valladares considered that the death of the animal in all probability was due to T. theileri because of the absence of piroplasma in its blood.

Pease [1908] doubted whether *T. theileri* gave rise to any disease in cattle because it appeared to him from investigations and enquiries that that trypanosome is fairly common amongst cattle and buffaloes, and the true nature of the disease caused by it is difficult to decide. Sowerby had shown Pease trypanosomes identical with *T. theileri* in the blood smears of buffaloes that were dying in Bombay but unfortunately no symptoms of these cases were recorded.

In December 1930, a bull (Ongole breed) belonging to the Madras Corporation was reported sick some time after serum-virus method of inoculation. The blood smears revealed a very large number of T. theileri (Plate XXX, Fig. 1) and the animal died with symptoms similar to piroplasmosis. There were no piroplasma in the blood smears nor did the blood of this bull produce piroplasmosis in two experimental calves into which it was injected. The experimental calves showed T. theileri in their blood for a few days and then the organisms disappeared. The bull showed symptoms exactly similar to those described by Valladares. It would appear therefore that under certain conditions, which we do not know definitely, these trypanosomes cause death in cattle. It is of interest that other bulls and bullocks that were with this animal in the sick line, did not show any T. theileri in their blood, though large numbers of stomoxys flies were present in the shed. Some of the stomoxys caught in the shed showed a large number of these trypanosomes in their gut (Plate XXX, Fig. 2 and Plate XXXI, Fig. 4). Some of these flies were kept alive for over a week and dissected. Only in a very few flies, well preserved but apparently dead trypanosomes were found. There were no developmental forms such as crithidia, etc., in them.

The finding of *T. theileri* in the blood of that bull gave an opportunity to study the parasites in blood smears and also in cultures. Cultures were successfully obtained in blood and glucose broth, N N N medium and also in Ray's medium which he used for culturing Leishmania. In all these media crithidia and leishmania forms were met with. The growth appeared as a white or dull grey speck on the surface of the blood cells in blood broth mixture and as dull white scales on the Ray's medium. There were present more of the leishmania forms on Ray's medium than in the fluid media or condensation water of N N N medium, in which mostly the crithidia forms prevailed.

The study of the crithidial forms showed that they resembled the ones found in tabanus flies—C. tabani Patton, 1909. The dimensions varied from 12 to 40  $\mu$  in length and 2 to 6  $\mu$  in width. Multiplication in rosette formation was also fairly common in the culture media. The leishmania forms were fairly large, 4 to 6  $\mu$  in diameter (Fig. 3, Plate XXXI). It is interesting to find that the curves representing the percentage of the crithidial forms in culture, in respect to their lengths, are almost identical with those of *Crithidia tabani* (Fig. VI). If the

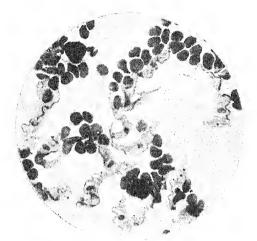


Fig. 1. T. theileri. Blood smear of bullock.

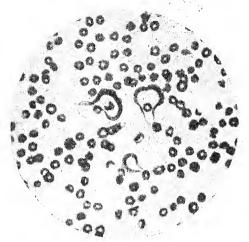


Fig. 2. Smears of blood from the mid gut of stomoxys soon after feed.

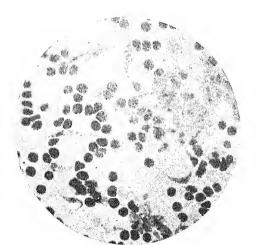


Fig. 3. T. theileri. Blood agar.

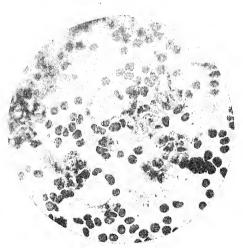


Fig. 4. T. theileri. Culture in blood broth.

## PLATE XXXI.

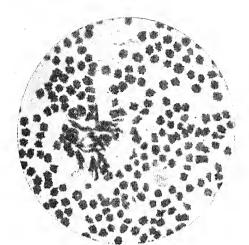


Fig. 1. T. theileri.
Bunch of crithidia—Blood broth.

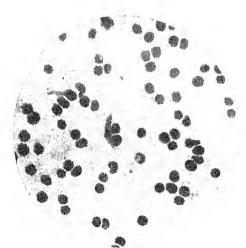


Fig. 2. T. theileri. Crithidia. Culture in blood broth.

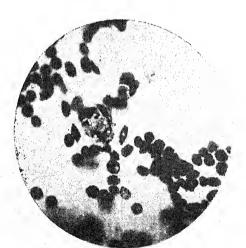


Fig. 3. T. theileri, large. Leishmania forms dividing (Blood agar.)

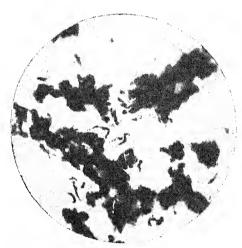
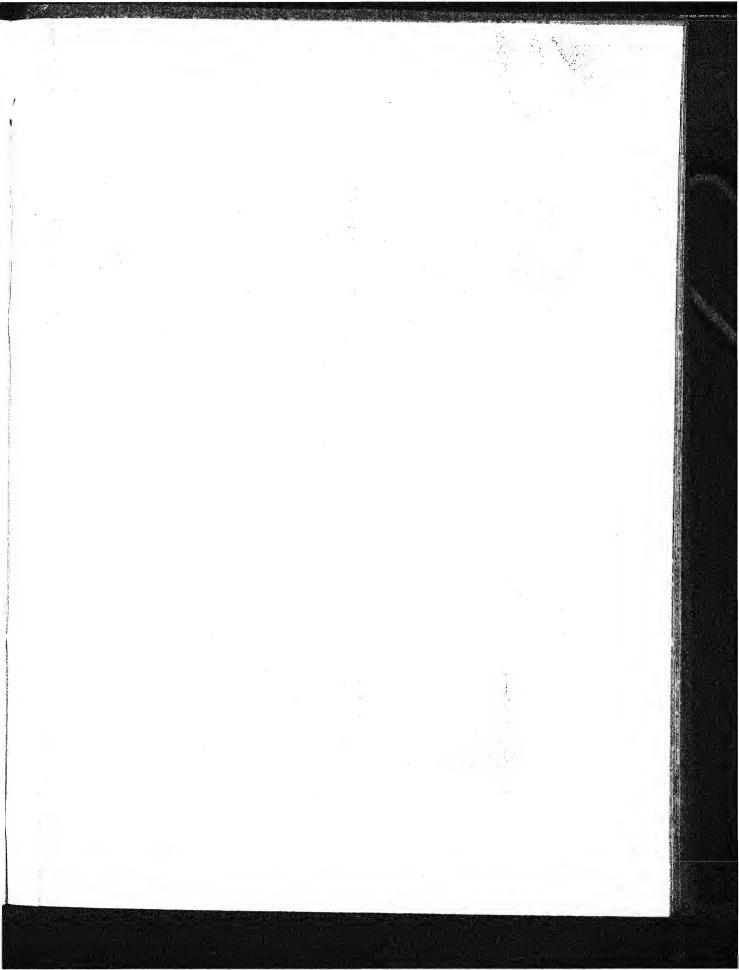


Fig. 4. Section of mid gut of stomoxys. Seven days after feed.

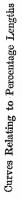


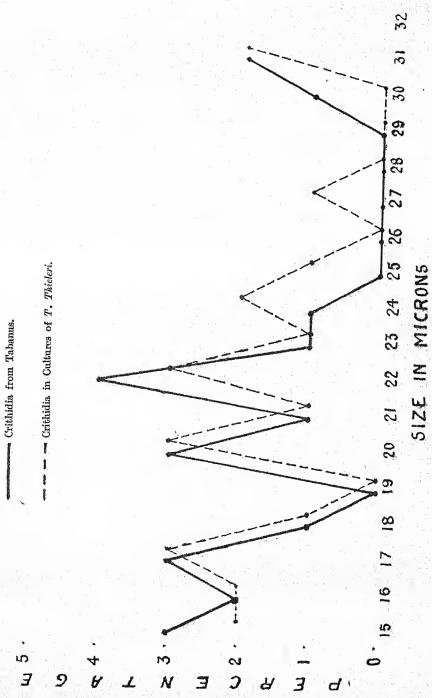


Cultural forms of T. theileri.

33

Fig. VI.





cultural changes represent the true developmental cycle, then there appears to be some relationship between *crithidia tabani* and the cultural forms of *T. theileri*.

Noller [1916] concluded that Crithidia subuluta Ledger, 1904, from the gut of Tabanus glaucopis is the developmental form of T. theileri. Wenyon [1926] suggests that if Crithidia subuluta is the insect phase of T. theileri, the other similar flagellates of tabanidae and their allies may also be the developmental form of the trypanosomes. Noller [1925] appears to have established this identity in the case of crithidia from Haematopota pluvialis. He injected clean calves with cultures of flagellates of that fly and recovered T. theileri in the blood cultures of those calves. We have said above that we injected crithidia found in tabanus flies eaught in Kaveripakam, into calves with negative results. In nature, if cattle are to get infected with T. theileri, it should be with developmental forms directly from an insect vector. Since the method employed by us is direct from the fly to the calf through the needle, as done by Noller, it is not understood why our experiments failed to produce T. theileri in the calves. One calf was drenched with the crithidial forms of T. theileri in culture. Even this calf failed to show the trypanosomes in blood cultures made from it.

During the past three years it has been possible to culture the blood of over 200 virus producing calves, and the blood of only one calf showed the cultural forms of *T. theileri*. In one calf recently, *T. theileri* was seen in its blood in fairly large numbers a few hours before it died of rinderpest.

In this Laboratory during the year 1928-29, *T. theileri* has been recorded in the blood of a few calves used for rinderpest virus production, in a cow in a dairy at Coonoor and a calf at Madanapalli [Rao and Ayyar, 1931]. The blood culture experiments show that an extremely small percentage of animals show *T. theileri* and it may be said that these parasites are not commonly found in bovines, though it is possible that the regional distribution is wide. Our experimental work also shows that it is doubtful if *C. tabani* have any relation to *T. theileri*.

#### SUMMARY.

Experimental evidence shows that the smaller trypanosomes found in cattle, dogs and ponies in S. India are identical with T. evansi.

The small trypanosomes found in cattle are pathogenic to borses when inoculated to them.

There appears to be little experimental evidence to prove that cattle and buffaloes form reservoir hosts of *T. evansi*.

T. theileri is occasionally met with in the blood of cattle in South India.

The morphology of the cultural form of *T. theileri* appears to be identical with the forms met with in the gut of *T. theileri*, but the identity could not be confirmed by animal inoculation.

Acknowledgments. It is our pleasant duty to thank Mr. P. T. Saunders, O.B.E., M.R.C.V.S., Director of Veterinary Services, Madras, for obtaining sanction of runds to conduct experiments; and to Mr. T. J. Hurley, M.R.C.V.S., D.V.S.M., for the facilities given to us. Our thanks are due to Mr. S. K. Sen, Protozoologist, Muktesar, for the help in getting at some references and to Mr. K. Kylasamier, Superintendent, The Serum Institute, Madras, for making it possible for us to obtain blood from virus producers for cultural tests. Our thanks are also due to the College Artist, Mr. P. Doraiswamy Mudaliar for the illustrations made for us.

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ources.

Date of 6th reinfection and Strain; No. of days after last reinfection.

nd September 1933 quine Strain from Dog 527. (4 days.)

nd September 1933 ulne Strain from Dog, 527. (4 days.)

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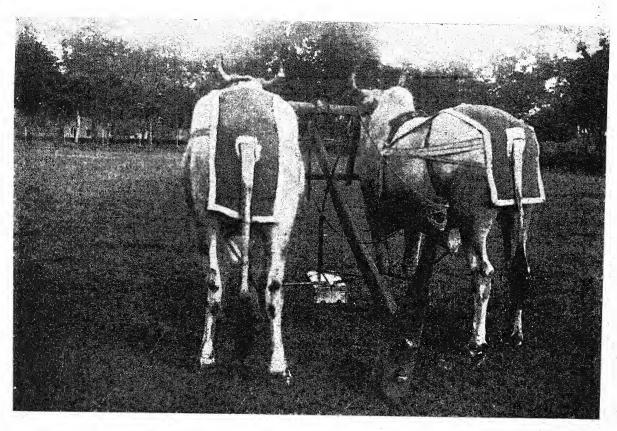
in September 1933 ulne Strain from Calf and Dog, 527. (10 days.)

id September 1933 quine Strain original from Dog, 527. (4 days.)

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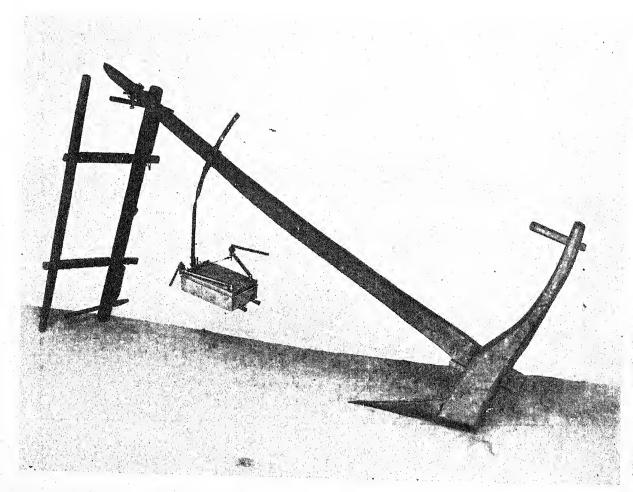
Result.	Date of 7th reinfection and Strain; No. of days after last reinfection.	Result.	Date of 8th reinfection and Strain; No. of days after last reinfection.	Result.	Date of 9th reinfection and Strain; No. of days after last reinfection.
			÷		
	-		·	*	
—ive.	10th October 1983 Equine Strain Relapse from Dog, 527 (W. R., 562). (19 days.)	ive.	30th October 1933 Bovine Strain from Pony, 519, Massive dose. (20 days.)	—ive.	Died due to other causes on 6th November 1933. Received 1 c.c. of Oleum Olivae intraperitoneally on 26th August 1933 with an attempt to break
Died due to	o other causes on 23rd Septer	nber 1934.			an attempt to break down the immunity.
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—ive.	22nd September 1933 Equine Strain original from Dog, 527 (4 days.)	-ive.	Died due to other ca	uses on 1st Oc	tober 1983.
—ive.	22nd September 1933 Equine Strain original from Dog, 527. (4 days.)	+ive.	Trypanosomes present died on 3rd (	t on 30th Sep October 1933,	tember 1933 and III.
-ive.	Died due to other e	auses on 30th	September 1922		
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PLATE XXXIV.



Complete arrangement of the apparatus when the plough is in operation.

PLATE XXXIII.



Skeleton attachment of the apparatus to the beam of the plough.

# AN APPARATUS FOR COLLECTING URINE OF BULLOCKS AT WORK.

BY

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AND

## AKBAR ALI B.Sc. (AGRI.)

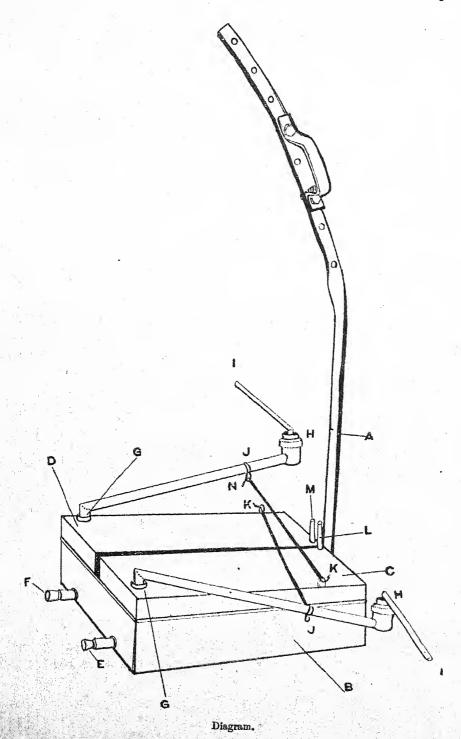
Agricultural College, Lyallpur.

(Received for publication on 14th August 1934.)

(With Plates XXXIII and XXXIV and 1 text-fig.)

In a previous publication [ Lander, 1926 ] a description has been given of the harness used for collecting dung and urine from bullocks and cows, whilst under experimental metabolism trials. The harness there described is suitable only for animals when stationary and cannot be employed for a bullock, when working in the field. In this paper harness and apparatus are described which can be employed for collecting dung and urine without loss, while bullocks are actually at work with a plough or other implement. It is essential in such an apparatus that urine shall not collect in the sheath bag but pass automatically, whatever may be the position of the bullock, into a container attached to the plough, from which it may be drawn off at intervals during the working period without any loss, or derangement of the apparatus.

The contrivance now described is depicted in Plate XXXIV which shows the complete arrangement when the plough is in operation. Plate XXXIII shows the skeleton attachment to the beam of the plough, and the diagram shows the essential features of the apparatus. This consists firstly of a metal beam 'A' which can be attached to the beam of the plough and admits of adjustment by means of bolts as shown.



The approximately vertical metal beam 'A' is bent roughly at a right angle at its lower end and slides into a groove on the lower surface of a metal tray.

Fitting into this metal tray 'B' are two metal boxes 'C' and 'D', at the lower ends of which are two brass projections 'E' and 'F' which pass through holes in the supporting tray 'B', and which can be closed by rubber stoppers. At the top of each of these two boxes there is an orifice shown at 'G' into which a brass tube 'G-H' fits. Free circular movement is permitted to the tube 'G-H' round the orifice 'G', the tube being prevented from travelling on to the top of the main receptacle by means of a small flange shown at 'N'. At the position 'H' there is a 'ground-in-joint' by means of which the tube 'H-I' can be removed, and by means of which also full play is permitted according to the movement of the bullock, the arm 'H-I' being capable of a free circular movement. The joints at 'G' and 'H' are, therefore, flexible and permit the whole of this arm from 'G' to 'I' as a whole to move round an arc or each part of the arm separately. In order to increase the play of this arm as the bullock moves round corners and in order to bring back the arm 'G-H' as the bullock returns to its position parallel to the beam of the plough a rubber strip 'J-K' is attached as shown.

The urine bags attached to the sheaths of the bullocks have short rubber tubes attached to them (Plate XXXIV) and these in turn are attached at their distant ends to the brass tubes at 'I.' Each of the two boxes which are placed in the tray has a capacity of 4 litres, and when the bullock urinates, urine passes directly via the rubber tube along the path 'IHJG' into the box and can be removed at convenient intervals by taking out the rubber stoppers at 'E' and 'F'. The brass tubes are nickled inside to prevent rusting. 'M' and 'L' are air holes. It will be seen that when the bullock is walking close to the beam of the plough the brass tube from 'G' to 'H' is parallel with the animal's body, and as the bullock moves away from the beam the combination of joints at 'G' and 'H' together with the rubber bands tend to take this tube at right angles to the animal's body.

The apparatus works with complete satisfaction, it is very light and neither interferes with the work of the bullocks nor touches the ground. Urine does not collect in the sheath bag, but is immediately conducted to the receptacle without loss through leakage or splashing.

The dung is collected during the course of work in the usual water proof bags as shown in the Plate XXXIV.

REFERENCE.

Lander, P. E. (1926). Agric. J. Ind. 21, 347.

## A CASE OF MONSTER AT THE GOVERNMENT CATTLE FARM, HISSAR.

### BY S. M. A. SHAH, M.R.C.V.S.,

Assistant Superintendent, Stock, Government Cattle Farm, Hissur.
(Received for publication on 29th September 1934.)
(With Plates XXXV and XXXVI.)

The term "monster", "monstrosity" or lusus naturae, is applied to a creature which presents some serious anomaly or organic deviation in form or structure or both, in one or more parts of the body.

#### ORIGIN OF MONSTROSITIES.

In ancient times the appearance of monstrosities were ascribed to the influence of enraged gods, and they were regarded with fear and horror; or they were looked upon as prodigies or freaks of nature, and described as marvels or curiosities. The opinion which at present prevails with respect to these malformations is to the effect that the embryo or foetus has been submitted to some kind of alteration in utero, and that this has been produced during the interval between conception and birth.

Meckel and others have shown the striking analogy there exists between many anomalies and various transitory conditions of embryonic organization. Saint Hilaire has proved experimentally, by means of eggs artificially hatched, that the production of monstrosities is due to the interruption or accidental suspension of development which had commenced in a regular manner.

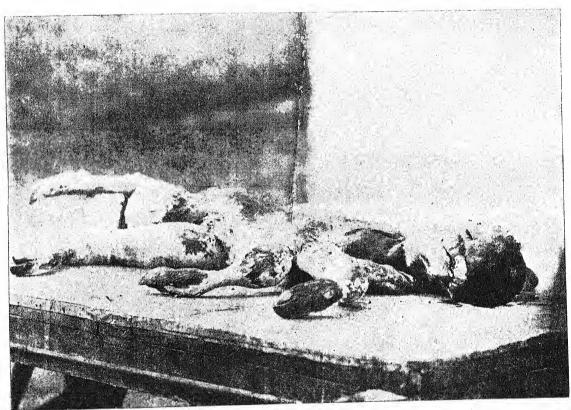
#### FREQUENCY OF MONSTROSITIES.

Gurlt gives the following list of 740 monstrosities showing the proportion in each species: cow 239, ewe 179, sow 87, bitch 78, cat 71, mare 56, goat 24, mule 3, ass 3. Saint Cyr in 71 instances found 45 calves, 16 lambs and kids, 4 pigs, 4 kittens, 1 puppy, 1 foal. Baumeister and Rueff mention that in the king of Wurtemberg's private stud, of 2,340 foals produced, there were only 9 monstrosities. In the Hohenheim dairies, among the cows the monstrosities were only one-half per cent; and among sheep one in 768.

At the Government Cattle Farm, Hissar, since 17th May, 1933, out of 1,574 calvings this is the only case that has been observed. The pedigree and breeding history of this cow along with her sister No. 79-H-2/1 is attached. The breeding history of her mother No. 295 H 4/10 is also attached.

#### HISTORY.

Cow No. 255 H 2/25 was brought to the hospital on the morning of 7th June 1934. She was showing all the symptoms of a case of dystokia. On examination the (402)



A case of monster at the Hissar Cattle Farm.

## PLATE XXXVI.



A case of monster at the Hissar Cattle Farm. Head circumference 27''.

toetus exhibited thigh and croup presentation. The cow was exhausted and expired after a few hours and on post mortem a monster was found in the uterus.

## DESCRIPTION OF THE MONSTER.

The body of the monster consisted of a flabby mass devoid of hairs, although here and there patchy hairs were seen. The dermal coat was not fully developed. The legs were rather massive especially the joints. The texture of the muscles resembled that of fat. The marked feature of the monster was its head, which resembled the shape of a bull dog's head. Hydrocephalus was well marked. The bones of the cranium were not fully ossified and were soft in texture. On the cranium there was a bushy growth of long hair and the usual sleak coat was missing. The eye lids were prominent and tumor-like in appearance. The ears were long, broad and fleshy. The nose was prominent, broad and massive, giving the appearance of a bull dog face. The upper jaw was more or less missing, with two rudimentary teeth hanging. The lower jaw was imperfectly developed protruding forward under the upper jaw, with a well marked tooth. The characteristic feature was the total absence of the tongue. The cheeks were rounded and massive, more like human cheeks. The description denotes that it was a typical case of Schistocephalus-hydrocephalocele.

The author has gone through the records of this Farm and this seems to be the first case of the disease recorded there.

Two photographs of the monster (Pl. XXXV and XXXVI) were taken:

- 1. Full body.
- 2. Head.

	1.71
Cow No. 295-4/10 gave the following f	emale stock—
Cow No. 299—4/10 gave one 156.	
<ul> <li>(1) F. Produce No. 156-H-2/7</li> <li>(2) F. Produce No. 197-H-3/9</li> </ul>	She was transferred to cows in 22nd November 1922 and was sold to R. M. Fazal
(2)	Dad Khan on 27th February 1924 Without
	giving birth to any calf.  She was transferred to cows on 17th Octo-
(3) F. Produce No. 79-H-2/1	ber 1924 and was auctioned on 9th April 1934. Her pedigree sheet and breed his-
	tory are enclosed.
D I 249 H-3/2	Sold to a zamindar of Amritsar District on
(4) F. Produce No. 248-H-3/2	14th January 1926.  She was transferred to cows on 21st May
(5) F. Produce No. 255-H-2/2	1928. Her pedigree as Well as bicoming
	history are enclosed.

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Breeding	history	of	cow	No.	255-2/25.
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12. 72.11		Sex of calf.	Brands of the calf.			Disposal of the ealf.
Served by Bull No.	Date of calving.		Brand No.	Farm Brand.	Age Braud.	_
\$35-H-4/0	11th December 1928	Female	525	Н	1.8	Transferred to cows on 1st January 1932.
334-H-3/5	2nd July 1930 .	Male	253	Н	3.0	D. B. Amritsar on 18th July 1933.
250-H-3/6	7th June 1932 .	Male .	300	н	2.2	>7.

## Breeding history of cow No. 79-H-2/21.

Served by		Sex of	Brands of the calf.			Disposal of the calf.
Bull No.	Date of calving	calf.	Brand No.	Farm Brand.	Age Brand.	
45-H-1/9	21st May 1925	Male	255	н	25	D. B Lahore on 16th Nevember 1928.
155-H-2/7	18th November 1926	Female	389	II .	46	To Cows on 10th October 1929.
108-H-2/0	7th January 1928 .	Male .	·		· · · · · · · · · · · · · · · · · · ·	Died of Navel ill ou 11th January 1928.
465-H·4/7 .	14th November 1928	Mule	570	н	1.8	D. B. Gurgaon on 9th November 1933.
334-H 3/5	24th June 1930 .	Female	109	н	2.0	To Cows on 31st May 1933.
48·Η. Λ. <b>-1</b> /6	22nd November 1931 .	Male .	443	Н	4.1	D. B. Delhi on 19th March 1934
135-H-1/¥	27th February 1933	Male .	83	н	1.3	

# Government Cattle Farm, Hissar. Pedigree Form 2/1. Name Date of Birth—3rd June 1921

.Dam.	etation yields.	Sire.	-	
295-H-4/	10.	176-E	1-3/9.	
54-H-1/02.	39-H-1/2.	311-H-3/1.	130-H-3/4.	
	73-S-2/3.		485-G-4/5.	
		ttle Farm, Hissar.  see Form.  Date of Birth—11th April 1925. Colour-  Sire.  161-H-2/0.		

295-H-4/I	0.	161-1	1-2/0.
54-H-1/U2.	54-H-1/U2. 39-H-1/2.		66-H-1/5.
	73-5-2/3.	28-H-1/7. 39-H-1/2.	317-H 4/9. 258-H-4/8.

## **ABSTRACTS**

Filtrable Virus Carriers. Gibbs, C. S. J. Infect. Dis. 53, 169-174. (1933).

From the data collected personally in China and in the United States of America, representing the culmination of 12 years of field observations, inspection of meat, post-mortem examination and laboratory studies, the author records the conclusion that chronic carriers of the filtrable viruses are realities and not merely possibilities. The article relates to hog cholera, infectious laryngo-tracheaitis of poultry and rinderpest, and it is claimed that virus-carriers have been identified by laboratory tests and not merely as possibilities through a posteriori reasoning:—

Hog Cholera. In 'runt' hogs which had passed through the disease longtime ago, maximum period being 94 days, certain button-like ulcers on the gastric and intestinal nucous membranes were found at autopsy, and normal animals were free from these lesions. Active lesions from the large intestine, showing the diphtheritic and typical appearance of hog cholera, and being relatively free from secondary micro-organisms, were chosen. The material was ground with sterile sand in sterile mortars, and the coarse particles including sand were removed by filtration. Subcutaneous inoculation into 12 healthy and unexposed pigs, 4 weeks old, was made, and of these ten developed the disease. Carrier cases showed an uncertain appetite, persistent diarrhoea followed by emaciation and stunted appearance. Of the 261 hogs examined, five of six suspected carriers were proved to harbour the virus.

Infectious Laryngo-tracheaitis. Details are given of how the swabs from the trachea of suspected carriers were employed to infect susceptible chicken, the disease being readily reproduced in the majority of cases.

Rinderpest. By an arrangement with a butcher tissues were collected from seven cases out of 200 cattle, in which the chronic carrier stage was suspected. Pyloric ulcers were triturated in sterile saline solution and inoculated into susceptible calves 6 weeks old. Four of the seven cattle were thus proved to be carriers, up to 177 days after recovery from all outward appearances of the disease. In the author's experience chronic carriers of rinderpest enjoyed good health and passed as sound animals in the market.

This article has been written in the belief that no careful investigation had hitherto been made to prove the existence of carriers of the common filtrable virus diseases. (Cf. Ind. J. Vet. Sc. Ani. Hush. 1932, 2, 357-382) [S. D.]

(1) Experimental Studies of post-mortem bacterial invasion in animals. Burn, C. G. J. Infect. Dis. 54, 388-394. (1934).

## (2) Post-mortem bacteriology, J. Infect. Dis. 54, 395-403.

To evaluate the results of cultural examination upon tissues and organs at autopsy, and to find out the relative importance of factors governing the types of bacteria and their spread through the body after death, the author experimentally inoculated suspensions of B. coli and Staphylococci into the various tissues and body cavities (pleural cavity, oral cavity, intestines subcut tissues) of healthy guineapigs and rabbits, killed by cardiac puncture, and attempts were then made to isolate the bacteria from the internal organs after keeping the animals at various temperatures for different periods. The experiments were controlled by observations on animals into which no bacteria were injected, the conditions of the experiment being otherwise similar.

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B coli and staphylococci were chosen since these organisms were the most frequent organisms to be isolated from human post-mortem material. Strains of other organisms injected in overwhelmingly large quantities, produced only negative results even though special opportunity, increased temperature, etc., was given to aid the invasion. The failure of staphylococci to invade within five hours, in contrast to the invasion of B. coli within the same period, illustrated the difference in growth response of different kinds of bacteria in the environment. The experiments showed that the organisms found in the bronchi and lungs reach there from the mouth through the traches. Anaerobic sporeforming bacillus, e.g., Cl. Welchii, has been shown to be capable of penetrating the mucosal lining of the intestine, and the isolation of this organism indicates the intestines to be the probable source, whereas the aerobic execi and B. coli failed to invade the intact membranes of the respiratory and intestinal tracts.

(2) In the second article data have been presented to show (a) that the lungs, kidneys, liver, spleen and heart blood gave frequency of growths in the order named, (b) that B. coli and staphylococi, streptococci nonhaemolyticus and Cl. Welchii are the most frequently isolated strains, while the other types isolated are generally associated with disease processes in the body.

Further information as to the principles governing the multiplication of bacteria in the tissues after death, such as autolytic and PH changes, are promised in a subsequent paper. [S. D.]

Histological changes in the Central nervous system following equine encephalomyelitis. (With two plates) Larsell, O., Harring, C. N., and Meyer K. F. Am. J. Path. 10, 361-373. (1934).

The brain and spinal cord of 8 horses, infected with encephalo-myelitis virus by contact or by experimental inscalation were studied histologically, and the most constant lesion was found to be perivascular entits containing lyphocytes, polymorphs and monocytes. Nerve cells in the neighbourhood of blood vessels showed more extensive degeneration than elsewhere, suggesting that the virus is conveyed in the vessels or along their sheaths. Experimentally infected guinea pigs and virus-affected human beings also showed the perivascular infiltration. Of the controls, however, three horses, which succumbed to sepsis, and another which was destroyed, failed to show the perivascular re-action.

The virus infected animals revealed inclusions in numerous nerve cells. In an apparently normal horse of 12 years of age, and in 3 others that died of sepsis, the cell inclusions were present in small numbers only. The author therefore concludes that in any case the pathological effect of encephalomyelitis appears to have increased the number of cell inclusions. The technique employed has been described in detail. [S. D.].

## The chemistry and pharmacology of Lathyrus peas. Stockman, R. J. Hyg. 34, 145-153. (1934).

Previous work showed that the symptoms of lathyrism in man and animals are due to salts of an acid contained in the pass, independently of any deficiency condition. Detailed account of the chemistry of the peas, and of the toxic actions of organic and inorganic salts prepared from them are now given. The results given in this article have a wide and general bearing since the toxic principle, acid salts of phytic acid, have been recovered from all cereal and leguminous seeds.

The general conclusion is that when given orally the poisonous organic phosphates contained in the peas are resolved by the bacterial flora of the bowels into the much less toxic inorganic alkaline phosphates. When the animal's system fails to dispose effectively of further quantities, toxic effects

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To evaluate the results of cultural examination upon tissues and organs at autopsy, and to find out the relative importance of factors governing the types of bacteria and their spread through the body after death, the author experimentally inoculated suspensions of B. coli and Staphylococci into the various tissues and body cavities (pleural cavity, oral cavity, intestines subcut tissues) of healthy guineapigs and rabbits, killed by cardiac puncture, and attempts were then made to isolate the bacteria from the internal organs after keeping the animals at various temperatures for different periods. The experiments were controlled by observations on animals into which no bacteria were injected, the conditions of the experiment being otherwise similar.

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B coli and staphylococci were chosen since these organisms were the most frequent organisms to be isolated from human post-mortem material. Strains of other organisms injected in overwhelmingly large quantities, produced only negative results even though special opportunity, increased temperature, etc., was given to aid the invasion. The failure of staphylococci to invade within five hours, in contrast to the invasion of B. coli within the same period, illustrated the difference in growth response of different kinds of bacteria in the environment. The experiments showed that the organisms found in the bronchi and lungs reach there from the mouth through the trachea. Anaerobic sporeforming bacillus, e.g., Cl. Welchii, has been shown to be capable of penetrating the mucosal lining of the intestine, and the isolation of this organism indicates the intestines to be the probable source, whereas the aerobic eocci and B. coli failed to invade the intact membranes of the respiratory and intestinal tracts.

(2) In the second article data have been presented to show (a) that the lungs, kidneys, liver, spleen and heart blood gave frequency of growths in the order named, (b) that B. coli and staphylococci, streptococci nonhaemolyticus and Cl. Wetchii are the most frequently isolated strains, while the other types isolated are generally associated with disease processes in the body.

Further information as to the principles governing the multiplication of bacteria in the tissues after death, such as autolytic and PH changes, are promised in a subsequent paper. [S. D.]

Histological changes in the Central nervous system following equine encephalomyelitis. (With two plates) Larsell, O., Harring, C. N., and Meyer K. F. Am. J. Path. 10, 361-373. (1934).

The brain and spiral cord of S horses, infected with encephalo-myelitis virus by contact or by experimental incentation were studied histologically, and the most constant lesion was found to be perivascular entiles containing hyphocytes, polymorphs and monocytes. Nerve cells in the neighbourhood of blood vessels showed more extensive degeneration than elsewhere, suggesting that the virus is conveyed in the vessels or along their sheaths. Experimentally infected guinea pigs and virus-affected human beings also showed the perivascular infiltration. Of the controls, however, three horses, which succumbed to sepsis, and another which was destroyed, failed to show the perivascular re-action.

The virus infected animals revealed inclusions in numerous nerve cells. In an apparently normal horse of 12 years of age, and in 3 others that died of sepsis, the cell inclusions were present in small numbers only. The author therefore concludes that in any case the pathological effect of encephalomyelitis appears to have increased the number of cell inclusions. The technique employed has been described in detail. [S. D.].

# The chemistry and pharmacology of Lathyrus peas. Stockman, R. J. Hyg. 34, 145-153. (1934).

Provious work showed that the symptoms of lathyrism in man and animals are due to salts of an acid contained in the pass, independently of any deficiency condition. Detailed account of the chemistry of the peas, and of the toxic actions of organic and inorganic salts prepared from them are now given. The results given in this article have a wide and general bearing since the toxic principle, acid salts of phytic acid, have been recovered from all cereal and leguminous seeds.

The general conclusion is that when given orally the poisonous organic phosphates contained in the peas are resolved by the bacterial flora of the bowels into the much less toxic inorganic alkaline phosphates. When the animal's system fails to dispose effectively of further quantities, toxic effects

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are manifested, as in the case of acute poisoning of farm stock after a large feed of lathyrus. Individuals of the same species of animals show considerable variation in their ability to resolve the toxic salts. Administered variously these toxic phosphates produce a marked degeneration of the nerve cells, and of nerve fibres of the cerebrospinal and sympathetic nervous systems. Moderate doses cause torpor and depression, larger doses clonic-tonic spasms. The available evidence is that the toxic action is probably a direct one.

The result of analysis of undecorticated lathyrus peas grown in India has been given. A proprietary preparation *Phytin* has been found to be similar in chemical properties and toxic action to phytates. The former commercial product was given an extensive trial in the treatment of rickets and neurasthenic conditions with negative results. [S. D.].